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A COMPARATIVE STUDY OF LIQUID-BASED CYTOLOGY VERSUS CONVENTIONAL SMEARS IN FINE NEEDLE ASPIRATION CYTOLOGY OF BREAST LESIONS

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ABSTRACT

Background: FNAC is a simple, safe and cost-effective method and is one of the triple diagnostic triads along with clinical breast examination and radiology methods (mammography/ ultrasonography). Cytological diagnosis is very beneficial for the preoperative assessment of various breast lesions. The application of LBC technique along with conventional smear can strengthen the efficacy of diagnosing various breast lesions on FNAC.

Aim: Comparison of liquid-based cytology versus conventional smears in fine needle aspiration cytology of breast lesions. **Materials and Methods:** A prospective study of 50 cases conducted over a period of 16 months included all female patients referred to the cytology department for FNAC. Two different passes were given, the first pass was used to prepare conventional smears and second pass was used for LBC smears. **Results:** Among 50 cases, 38 (76%) were benign and 12 (24%) were malignant. The commonest benign lesion was fibroadenoma and malignant lesion was Infiltrating Ductal Carcinoma. The difference between LBC and conventional smears was statistically significant pertaining to background blood-debris ($p < 0.00001$), informative background ($p = 0.0004$) and cellular architecture ($p = 0.0075$) while it was not statistically significant for cellularity, monolayering, nuclear and cytoplasmic details The sensitivity, specificity, PPV, NPV and diagnostic accuracy of FNAC in both LBC and conventional smears were similar, being 80%, 100%, 100%, 95.23% and 96% respectively. **Conclusion:** LBC cannot replace conventional cytology but can be used as a

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supplement with the conventional method to minimize false negative cytology rate and to increase the diagnostic accuracy of breast lesions.

Keywords: Conventional smear (CS), fine-needle aspiration cytology (FNAC), liquid-based cytology (LBC).

INTRODUCTION:

In the evaluation of breast lesions, the two most important procedures, fine needle aspiration cytology (FNAC) and core needle biopsy are preferred methods over open surgical biopsies.[1] FNAC is a simple, safe, and cost-effective method and is one of the triple diagnostic triads along with clinical breast examination (CBE) and radiology methods (mammography/ ultrasonography).[2] The conventional method of FNAC needs to be mastered to obtain good specimens for reporting various lesions.[3] Conventional smears (CS), though important in diagnosing breast lesions, are very time-consuming and exhausting to screen the entire smear due to non-uniform slide preparation and fixation. The conventional smears show cell overlapping and overcrowding along with background obscuring elements, and air-drying artefacts which can lead to poor nuclear details and cell morphology.[4] Liquid-based cytology (LBC) technique can help to reduce the above difficulties. LBC has many advantages over conventional smears, it provides better cytomorphological features, and other factors such as drying artefact, overlapping of cells, obscuring background elements like blood, mucous, and inflammation have been decreased in LBC leading to a more accurate diagnosis.[2,5] In the LBC technique, the aspirates obtained by FNAC can be directly transferred into a container containing a preservative solution, and smears with thin layer of cells are obtained through membrane-based or sedimentation LBP technique.[3] The LBC technique was originally developed for gynaecological cervical smears. In 1996, it got approval from the Food and Drug Association (FDA).[6] Thereafter, this technique was used for non-gynaecological cytology (conventional and guided FNAC) along with body fluid cytology. The cytomorphological features of LBC smears vary from conventional smears in the following different aspects as follows: (1) The cells in each smear are a monolayered representative sample of the whole material that was collected in the LBC container. (2) It can lead to some changes in both cell architecture and background morphology.[7] The aspirate from different organs like lymph nodes, salivary glands, thyroid, breast, soft tissues, and bone can be processed through the LBC

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technique.[6] The application of the LBC technique along with conventional smear can strengthen the efficacy of diagnosing various breast lesions on FNAC.[5]

MATERIALS AND METHODS:

A prospective study conducted over a period of 16 months (March 2021 to June 2022).

Sample size: 50 cases

Study design: A prospective study.

Place of study: Cytology section of Department of Pathology, Mahatma Gandhi Mission's Medical College and Hospital, Kamothe, Navi Mumbai.

Inclusion criteria:

1. Female patients presenting with breast lump(s) who are referred for FNAC procedure.
2. Breast specimen received for histopathological examination.

Exclusion criteria:

1. Patients who are not willing to give informed consent for the FNAC procedure.
2. Uncooperative patients.
3. Males presenting with a palpable breast lump.

Procedure: A total of 50 cases were studied and had undergone clinical breast examination and FNAC procedure.

Clinical Breast Examination (CBE): All the patients were subjected to CBE and both the axillae were palpated for lymphadenopathy. The CBE findings were categorized into benign and malignant lesions.

FNAC Procedure: The process of sample collection for LBC and conventional smears of breast FNAC was done by a direct-to-vial method. During FNAC two different passes were given wherein, the first pass was used to prepare conventional smears and the second pass was used for LBC.

Direct smears prepared were fixed in 95% ethyl alcohol for at least 30 minutes or overnight and fixed smears were stained using PAP and H&E stain while air dried smears were stained with May Grunwald Giemsa (MGG) stain. The cytology smears were categorized into

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diagnostic categories (C1- C5) proposed by National Cancer Institute (NCI) in 1996.[8] Final cytology diagnosis was categorized into benign (category C2 and C3) and malignant (category C4 and C5) lesions.

Smears obtained by conventional, or LBC method were studied for the following cytological features and scoring was done for each cytological feature.[4, 6, 9]

Table 1: Cytological features and their scoring.

Cytologic features	Score			
	0	1	2	3
Cellularity	Zero	Scanty	Adequate	Abundant
Background: blood-debris	Zero	Occasional	Good	Abundant
Informative background	Absent	Present	-	-
Monolayer	Absent	Occasional	Good	-
Cell architecture	Non-recognized	Moderately-recognized	Well- recognized	-
Cytoplasmic details	Poor	Fair	Good	Excellent
Nuclear details	Poor	Fair	Good	Excellent

Cellularity was assessed and graded as follows:

• Zero - Duct epithelial cells are not seen on smear.

• Scant - Few number of groups of duct epithelial cells seen/ high power field (hpf)

• Adequate - Multiple groups of duct epithelial cells (8-10 ductal epithelial cells per group)

• Abundant - Multiple groups, clusters, and sheets of ductal epithelial cells with background showing bare nuclei and stromal fragments.

A total of 25 histopathological specimens were received in 10 % buffered neutral formalin and further subjected to grossing, tissue processing, paraffin embedding, sectioning, and staining by hematoxylin and eosin. The stained sections were studied for various histomorphological features and a final diagnosis was given. A cyto-histopathological correlation was done.

Statistical analysis: The collected data was stored and analysed using in Microsoft Excel and by using SPSS test of significance 'p' value was calculated. $P < 0.05$ was considered statistically

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significant. Cytological diagnosis was correlated with histopathological diagnosis and the specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated.

RESULTS:

Age-wise distribution: The age of the patients ranged from 13 to 76 years with a mean age of 37.48 years. The maximum number of cases were in the age group of 41-50 years (26%) whereas, the least number of cases were in the age group 71-80 years (2%) (Table No.2). Benign lesions were commonly seen in age-group of 21-30 years (11 cases) and malignant lesions in 41-50 years of age-group (7 cases).

Table 2: Age-wise distribution of all female patients.

Age group (Years)	Number of cases	Percentage (%)
11-20	09	18
21-30	11	22
31-40	09	18
41-50	13	26
51-60	03	6
61-70	04	8
71-80	01	2
Total	50	100

Side of breast lesion: Out of 50 cases, the left breast was involved in 25 cases (50%) and the right was involved in 24 cases (48%). Bilateral involvement of breast was seen in one case (2%).

Quadrant: Majority of the breast lump were seen in upper outer quadrant (23 cases) followed by upper inner quadrant (10 cases).

Duration of lump: 41 cases out of the total 50 cases presented with a duration of <5 months while 06 cases presented with a duration of more than a year.

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Size of breast lump: 37 cases (74%) presented with a breast lump of size <5 cm whereas 13 cases (26%) presented with a breast lump of size >5 cm.

Consistency: Majority of the cases (36 cases; 72%) were firm in consistency.

Breast Imaging Reporting And Data System Scoring (BIRADS): On ultrasonography, 37 cases were benign, and 13 cases were malignant. Maximum cases were with a BIRADS score of 2 (30 cases).

Cytological diagnosis and categorization on conventional and LBC smears: In the present study, on conventional smears, 36 cases were of category C2 (Benign), 8 cases of C5 (Malignant), and 2 cases were seen in each of Category 1, 3, and 4 (Table No. 3).

On LBC, 35 cases were of category C2 (Benign), 7 cases of C5 (Malignant), 3 cases were seen in each of Category 1 and 4, and 2 cases in category C3. (Table No. 3)

Table 3: Distribution of breast lesions according to International Academy of Cytology (IAC) category and comparison of various lesions diagnosed on conventional cytology and LBC.

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IAC Category	Conventional cytology	Number of cases	
		Conventional Smear	LBC
C1	Inadequate	2 (4%)	3 (6%)
C2	Breast Abscess/ Acute suppurative lesion	4 (8%)	5 (10%)
	Granulomatous Mastitis	4 (8%)	4 (8%)
	Cystic lesion	1 (2%)	1 (2%)
	Fibroadenoma	22 (44%)	22 (44%)
	Fibroadenoma with cystic change	1 (2%)	0 (0%)
	Fibrocystic lesion with secondary inflammation	1 (2%)	0 (0%)
	Benign proliferative breast lesion	3 (6%)	3 (6%)
C3	Proliferative breast lesion with atypia	2 (4%)	2 (4%)
C4	Suspicious of malignancy	2 (4%)	3 (6%)
C5	Epithelial Malignancy	7 (14%)	6 (12%)
	Mucinous Carcinoma	1 (2%)	1 (2%)
Total		50	50

Table 4: Association between cellular characteristics between Conventional cytology and liquid-based cytology.

Score	Cellular Characteristic	Total (n=100)	CS (n=50)	LBC (n=50)	Chi Square	P-value
0=Zero	Cellularity	4	2	2	2.365	0.50
1=Scanty		5	1	4		
2=Adequate		36	17	19		
3=Abundant		55	30	25		
0=Zero	Background - Blood Debris	26	1	25	36.519	<0.00001
1=Occasional		46	26	20		

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2=Good amount		12	8	4		
3=Abundant		16	15	1		
0=Absent	Informative Background	25	11	28	12.148	0.0004
1=Present		75	39	22		
0=Absent	Monolayer	13	8	11	2.616	0.2702
1=Occasional		28	10	15		
2=Good amount		59	32	24		
0=Non-recognized	Cell architecture	7	2	5	9.769	0.0075
1=Moderately recognized		21	5	16		
2=Well recognized		72	43	29		
0=Poor	Nuclear Details	6	2	4	5.722	0.1259
1=Fair		5	1	5		
2=Good		19	6	10		
3=Excellent		70	41	31		
0=Poor	Cytoplasmic Details	6	2	4	5.923	0.1153
1=Fair		6	1	7		
2=Good		18	8	07		
3=Excellent		70	39	32		

Cyto-histological correlation: On histopathological examination, the most common benign breast lesion was fibroadenoma (16 cases) and the malignant lesion was infiltrating duct carcinoma (3 cases). On comparison of conventional smears and LBC with histopathological diagnosis, 24 cases out of 25 cases showed concordance.

Table 5: Number of benign and malignant cases on histopathology

Lesions	Number of cases	Percentage (%)
Benign	20	80
Malignant	05	20
Total	25	100

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Table 6: Statistical analysis of conventional and liquid-based cytology.

Conventional and LBC cytology	Histopathological Diagnosis		Total
	Malignant	Benign	
Malignant	04 (TP)	00 (FP)	04
Benign	01 (FN)	20 (TN)	21
Total	05	20	25

TP: True positive FP: False positive TN: True negative FN: False negative

Table 7: Comparison of sensitivity, specificity, PPV, NPV and diagnostic accuracy of conventional cytology and LBC.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Conventional	80	100	100	95.23	96
LBC	80	100	100	95.23	96

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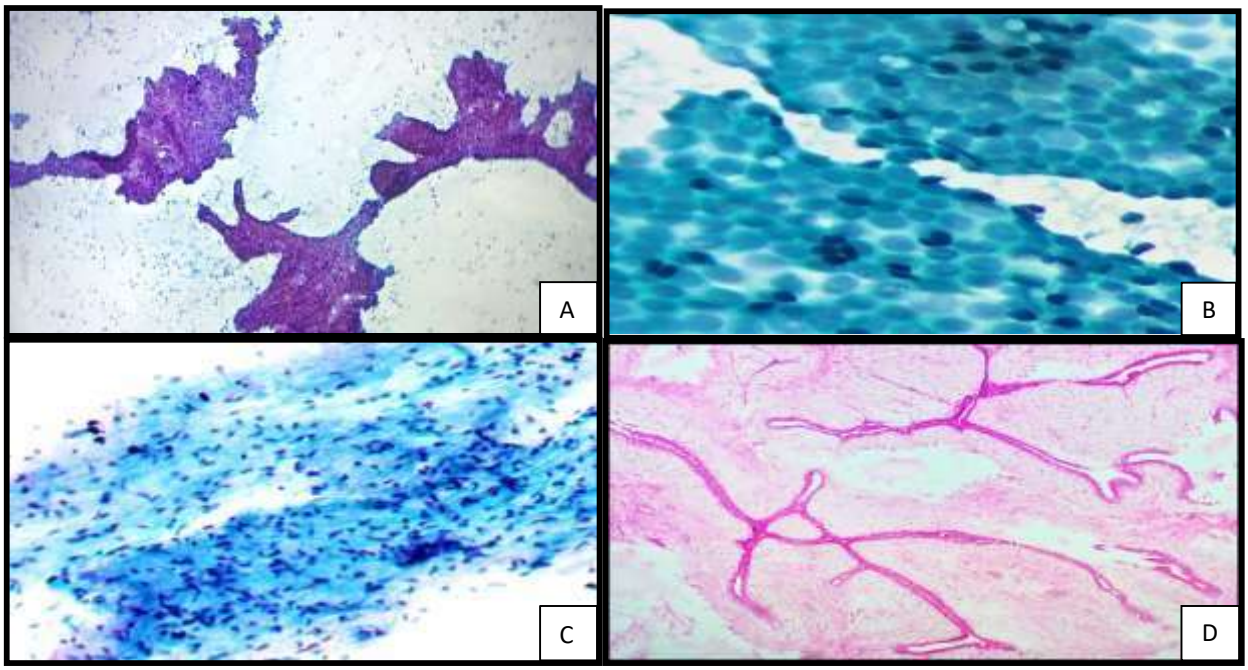

Figure 1: Fibroadenoma:- (A) Branching fragments (Antler-horn pattern) of ductal epithelial cells (CS)(PAP,x10); (B)Ductal Epithelial cells with dark nuclei of myoepithelial cells and few bare nuclei in background. (LBC)(PAP,x10); (C)Stromal fragments with spindle cells (LBC)(PAP,x40); (D) Fibroadenoma showing predominantly intracanalicular pattern (H&E)(x10)

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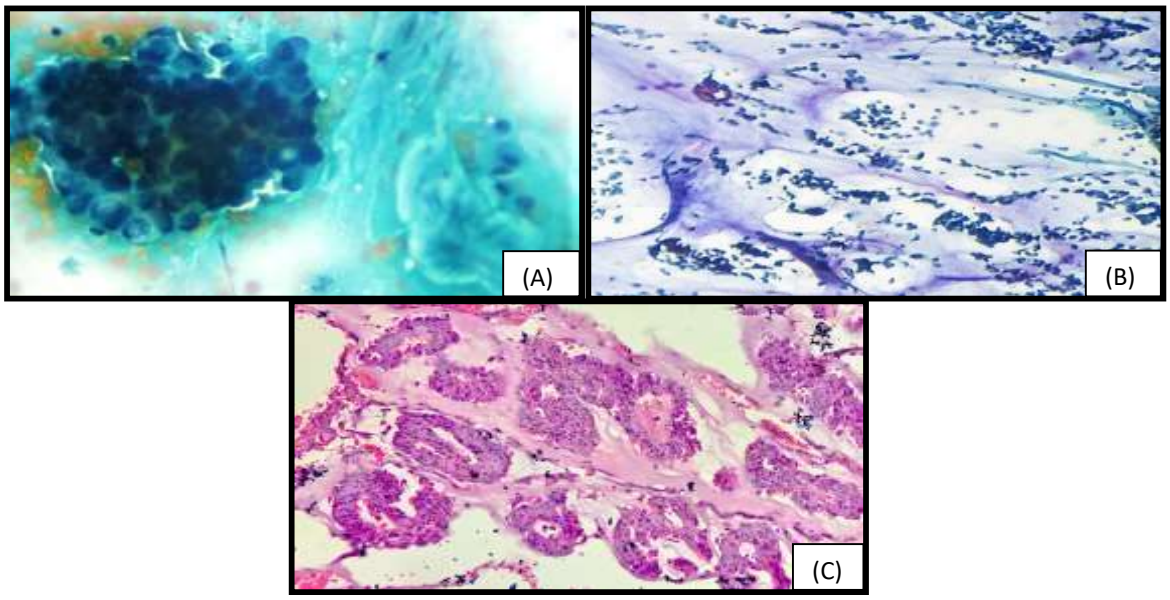

Figure 2: Mucinous Carcinoma:- (A) & (B) Ductal epithelial cell clusters showing moderate to marked anisonucleiosis, hyperchromaic nuclei, nuclear overlapping and overcrowding embedded in pool of mucin (A) (CS)(PAP,x40) & B) (LBC)(PAP,x10); (C) Clusters of tumour cells floating in pools of extra cellular mucin (H&E) (X10)

Discussion:

LBC is a technique that enables cells to be suspended in a monolayer making better morphological assessment possible. The material is collected in a liquid fixative which is later processed and followed by preparation and their evaluation. It was initially advised for gynecology samples but now is increasingly being used for non-gynecological cytology samples and FNA samples.[10]

FNAC plays a very important role in the evaluation of palpable breast lumps. It determines the nature of breast lesions with a high degree of accuracy.[10,11] However, the outcome depends upon the proper preparation of the cytology smears, how well-trained the individual who is performing the FNAC procedure is, and the experience of the cytopathologist who will be screening and finally interpreting the smears.[10,12]

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The aim of the study was to compare the diagnostic efficacy of liquid-based cytology versus conventional smears in the evaluation of various breast lesions and to observe the advantages and disadvantages of these two techniques.

A total of 50 female patients presented with a breast lump(s) and underwent an FNAC procedure. The age range in the present study was from 13 to 76 years with a mean age of 37.48 years and was comparable to findings observed by Mahinderu et al.[9] (15-70 years and a mean age of 35.55 years). Majority of the cases belonged to 41-50 years age group which was similar to findings reported by Bindhuja et al.[10] and Siddique et al.[13] The upper outer quadrant of the breast was affected more commonly (23 cases, 46%) followed by the upper inner quadrant (10 cases, 20%). Siddique et al.[13] also observed the upper outer quadrant to be commonly affected.

In the present series, 37 cases had an average size of > 5 cm, and 13 cases had a size of, more than or equal to 5 cm. Out of the 50 cases, all cases presented with a breast lump, 23 cases were associated with pain, 3 cases had fever, 3 cases with nipple retraction, 2 cases with nipple discharge, and 1 case presented with redness of skin.

The correlation of clinical breast examination, BIRADS score and cytological findings helped to improve the diagnostic accuracy of breast lesions. Maximum number of cases were of BIRADS score 2 (30 cases), followed by score 5(8 cases), score 3 (7 cases), and score 4 (5 cases). There were no cases with a BIRADS score of 1 or 6.

Comparison of cytomorphological features of conventional FNA smears and LBC smears:

Cellularity: In the present study, there was no statistically significant difference between LBC and conventional smears (p-value: 0.50). Various authors have stated that the cellularity of cytology smears depends on the number of passes given during FNA and the expertise of the individual performing the procedure.[2,12,14] We observed adequate cellularity in CS (47 cases) as compared to LBC smears (44 cases) which is similar to the observation made by Binduja et al.[10] where abundant cellularity was seen more in conventional smears (69.1%). Kumar et al.[15] in their study proposed that the reason for less cellularity on LBC could be due to loss of material during preparation and secondly, during the second pass, the needle must not have hit the representative lesion or due to hemorrhage after the first FNA pass.

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Background blood debris:

We reported more cases with a clean background in LBC smears as compared to conventional smears due to the elimination of obscuring elements such as blood, excessive inflammation, and cellular debris. We identified a statistically significant difference in background blood debris between LBC and conventional cytology (p-value < 0.00001). These findings align with similar observation made by other authors in their respective studies. [2,4,6,10]

Informative Background:

In FNAC, the informative background plays a crucial role in identifying various lesions. It is well preserved in conventional as compared to LBC. Our study revealed that the samples analyzed by LBC showed reduction in informative background. Gerhard et al.,[12] Perez-Reyes et al.[16] and Veneti et al.[17], and also found that the informative background was lost in LBC preparation. Few authors also found difficulty in diagnosing fibroadenoma on LBC preparations due to loss of stromal fragments, decreased cellularity, paucity of bipolar cells and myoepithelial cells.[12,16]

Monolayer:

There was no statistically significant difference (p-value = 0.2702) observed by us regarding the presence of monolayers of cells between LBC smears and conventional smears (Table No.4). We observed that on LBC the cells were tightly packed, present in small clusters, dispersed singly, and were smaller in size than on conventional smears. These features were also observed by Sharma et al.[4] in their study of 75 cases of breast lesions.

Cell Architecture:

In the present study, the cell architecture was better visualized on conventional smears and showed a statistically significant difference (p-value: 0.0075) (Table No.4), similar to findings observed by Bindhuja et al.[10] Whereas, LBC showed loss of cell architecture comprising fragmented shortened cellular fragments. Some authors have observed that the cell morphology and architecture were better assessed on LBC which showed less nuclear overlapping, less fragmentation of cells, and discohesive cells. [9,18,19,20]

Nuclear Details:

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Based on nuclear details, there was no statistically significant difference in the present study, which was similar to the observation made by Kord et al.[2] and Sharma et al.[4] We observed nuclear details to be comparable on both LBC and CS, which were similar to the findings reported by Kord et al.[2] However, other authors noticed better nuclear details on LBC compared to conventional smears.[6,9,10,21] A comparative study of Thin-layer and conventional preparation by Perez-Reyes et al.[16] noted a marked difference in nuclear size exhibiting shrinkage of nuclei on Thinprep.

Cytoplasmic Details:

Based on cytoplasmic details, this study showed no statistically significant difference regarding cytoplasmic features between LBC and conventional smears (p-value: 0.1153) (Table 4). These findings were comparable with the findings noted by Sharma et al.,[4] Perez-Reyes et al.[16] and Veneti et al.[17] Few authors have observed better cytoplasmic details on LBC than in conventional smears.[6,10,18,21,22] Bindhuja et al.[10] in their study noted that 80% of LBC smears depicted very clear cytoplasmic details compared to only 20% of conventional smears.

Cyto-histological correlation:

Out of the total of 50 cases, histopathology correlation was available in 25 cases. On comparison of conventional smears with histopathological diagnosis, we documented 20 cases of C2 category followed by 4 cases of C5 and one case of C3. Out of 25 cases, 96% of cases (24/25 cases) showed concordance with the conventional smear diagnosis while one case which was diagnosed as proliferative breast lesion with atypia (C3 category) was diagnosed as DCIS on histopathology.

On comparison of LBC with histopathological diagnosis, 96% of cases (24/25 cases) showed concordance while one case which was reported as a proliferative breast lesion with atypia (C3 category) was diagnosed as DCIS on histopathology. A case of acute inflammatory lesion on LBC was reported as a fibrocystic lesion with secondary inflammation on conventional smear and was later diagnosed as fibrocystic disease on histopathology. The reason for the misdiagnosis on LBC may be in the second pass, the person performing the procedure may not have hit the representative lesion.

On histopathology, 20 out of 25 cases were benign and fibroadenoma (16 cases) was the commonest benign lesion followed by granulomatous mastitis (02 cases). Infiltrating ductal

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carcinoma (03 cases) was the commonest malignant lesion followed by one case each of DCIS and Mucinous Carcinoma.

In the present study, 23 cases of fibroadenoma were diagnosed on CS, whereas on LBC, 22 cases were diagnosed as fibroadenoma and one case was diagnosed as a benign proliferative breast lesion. This may be due to a marked reduction in the number of bare nuclei, no stromal fragments with loss of informative background leading to difficulty in diagnosing Fibroadenoma.

Michael et al.[18] in their study found that diagnosing Fibroadenoma on LBC can be very challenging as they had observed a decrease in the number of myoepithelial cells, an increase in the number of small cell aggregates, and single intact cells with the fragmentation of monolayer branching sheets on LBC. Rossi et al.[7] stated that cytological features of fibroadenoma on Thinprep can lead to erroneous diagnosis of malignancy because of the following features: small cellular aggregates, increased cellular discohesion, prominent nucleoli, decreased numbers of myoepithelial cells and loss of stromal fragments. Ryu et al.[1] stated that prominent nucleoli are a potential diagnostic pitfall in fibroadenoma cases. Carniello et al.[23] in their study of diagnostic dilemmas in Thinprep have stated that the homogenization step in Thinprep which disperses the debris and leads to a random distribution of cells can destroy the cellular architecture and can be one of the causes of reduced number of epithelial cells clusters and staghorn pattern on Thinprep smears.

Breast carcinomas can be accurately diagnosed on LBC smears if the following features are present: loose clusters with varying degree of atypia, isolated atypical cells and the increase of mitotic figures.[2,6] On LBC, IDC can be characterized by the presence of clusters of malignant ductal epithelial cells displaying pleomorphic hyperchromatic nuclei and scant to moderate cytoplasm with absence of blood and necrosis in the background.

In our study, all the malignant lesions were correctly diagnosed on both conventional as well as LBC smears however, the LBC smears showed a loss of informative background of necrosis and blood debris in malignant lesions. Kord et al.[2] in their study reported that clean background on LBC smears eliminates hemorrhage, necrosis, and inflammatory cells and leads to a faster and easier diagnosis of breast carcinomas. Tripathy et al.[6] observed that diagnosis of IDC was better on LBC preparations than conventional smears as the nuclear features were clearer on LBC.

We reported one case of mucinous carcinoma which was accurately diagnosed on both conventional smears as well as LBC smears. On LBC we could diagnose mucinous carcinoma

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with ease due to the presence of mucinous background however, few authors in the literature have stated that it was difficult to give the diagnosis of this lesion on LBC due to the absence of mucinous background.[12,16,17,18]

Table 8: Advantages and Disadvantages of LBC

Advantages	Disadvantages
1. Cellular material is limited to small areas. 2. Less time-consuming. 3. Removes the obscuring background debris, and blood by filtration. 4. Less number of slides are required for the examination. 5. Easier for collection of samples. 6. Overcomes problems such as air-drying artifacts. 7. Uniform thin-layered distribution of cells with less cellular overlapping on a clean background. 8. The remaining material can be stored in the appropriate containers and the slides can be stored for up to 6 months. 9. Cell-block preparation can be prepared from LBC material and further immunohistochemistry studies can be performed.	1. LBC can add costs to the laboratory. 2. Prior training is necessary to avoid cytologic misinterpretation. 3. Important background elements such as inflammatory cells, necrotic debris, and mucin can be lost during the processing. 4. Produces certain cytomorphological alterations and artifacts. 5. Can lead to less clear chromatin details with prominent nucleoli and smaller cell size. 6. Intranuclear inclusions can be difficult to visualize.

Statistical Analysis:

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In the present study, sensitivity, specificity, positive predictive value, and negative predictive value were calculated for LBC and conventional smears keeping histopathological diagnosis as the gold standard (Table 6). The sensitivity, specificity, positive predictive value, and negative predictive value of both the LBC as well as conventional smears were 80%, 100%, 100%, and 95.23% respectively with the diagnostic accuracy for both modalities being 96% (Table 7).

Conclusion: Both techniques have their advantages and disadvantages with LBC improving the quality of the sample, saving time, and reducing the probability of false negative cytology results, however, altered cellular architecture and morphology coupled with the loss of informative background can lead to erroneous diagnosis while conventional smears are a quick, easy and inexpensive method but air-drying and spreading artifacts may be present.

LBC cannot replace conventional cytology but can be used as a supplement with the conventional method to minimize false negative cytology rate and further increasing the diagnostic accuracy of breast lesions.

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