# COMPARATIVE EVALUATION OF THE DIAGNOSTIC ACCURACY OF CONVENTIONAL SMEARS TO LIQUID-BASED CYTOLOGY FOR BREAST LESIONS

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## ABSTRACT

**Background:** For the evaluation and assessment of both non-gynaecologic and gynaecologic specimens, extensive use of LBC (Liquid-based cytology) has gained popularity in the recent past. Better assessment morphologically is possible owing to the monolayer cell suspension. Also, LBC allows assessment of nucleolar prominence, smaller cell size, fragmented cell clusters, lost, reduced, or altered background material.

**Aim**: The present study was conducted to comparatively evaluate the utility and efficacy of Liquid-Based Cytology over conventional smears for assessment of the breast lesion, and to assess their use as an alternative to the conventional preparation.

**Materials and Methods:** The present prospective clinical study included 374 female subjects of the FNAC (fine-needle aspiration cytology) obtained from subjects having palpable breast lesions. The second pass was given, whereas, the first pass was used for either LBC or conventional smear. Several criteria were used to compare representative LBC and conventional smear. Individual scoring of each criterion was done followed by statistical evaluation.

**Results**: The study results showed that statistically non-significant difference was seen between LBS and conventional concerning nuclear and cytoplasmic details, presence of monolayer,cytoarchitectural pattern, and cellularity, whereas, a statistically significant difference was seen for background blood-debris and informative background.

**Conclusion**: The present study concludes that LBC can be a promising tool for the cytology field in liquid-based cytology with the potential to reduce slides number screened for each case and also reduce the turn-around time.

**Keywords:** Breast Lesion, Conventional Smear, Cytology, Fine-Needle Aspiration Cytology, Liquid-Based Cytology

## INTRODUCTION

With the increase in awareness among the general population concerning the lump in the breast, it has been the most common presentation in the females visiting the Outpatient departments globally, and especially in India. The lesions in the breast are usually seen as abnormal imaging findings, discharge from the nipples, and/or palpable lumps in the breast.[1]

Breast lesions on the cytologic examination can be seen in different ways using cytology and have an important role in both diagnosis and screening of different lesions. A quasi-routine clinical procedure used for breast lumps is FNAC (Fine-needle aspiration cytology) which constitutes a vital part of triple assessment done for assessing the palpable breast lumps. It is a cost-effective, easy to perform, rapid, and accurate procedure for evaluating breast lumps and lesions.[2]

Owing to the non-uniform fixation and slide preparation, conventional smears, despite being useful for diagnosis, are time-consuming and tedious for screening. Poor cellular and nuclear preservation are seen due to associated features with conventional smears including air and blood drying artifacts, obscuring inflammation,overlapping cellular areas, and thick cellular areas.[3]

For the evaluation and assessment of both non-gynecologic and gynecologic specimens, extensive use of LBC (Liquid-based cytology) has gained popularity in the recent past. Better assessment morphologically is possible owing to the monolayer cell suspension. Also, LBC allows assessment of nucleolar prominence, smaller cell size, fragmented cell clusters, lost, reduced, or altered background material. LBC uses cell rinsing in a liquid media preservative where sample processing is done on an automated device despite making a smear allowing the cell suspension in a monolayer. LBC also is superior concerning even cellular distribution and rapid fixation in a small area on the slide. Hence, the time needed for assessing using cytopathologists and screeners is reduced. Also, the material that remained in fixative liquid can be utilized for immunocytochemistry ancillary studies.[4]

With the increase in the incidence of breast lesions globally and especially in India, and their potential to get cured, the present clinical study was conducted to comparatively evaluate the utility and efficacy of Liquid-Based Cytology over conventional smears for assessment of the breast lesion, and to assess their use as an alternative to the conventional preparation. The study also aimed to assess if LBS can be used as an alternative to conventional smears for assessing breast lesions.

## MATERIALS AND METHODS

The present prospective clinical study was conducted to comparatively evaluate the utility and efficacy of Liquid-Based Cytology over conventional smears for assessment of the breast lesion, and to assess their use as an alternative to the conventional preparation. The study also aimed to assess if LBS can be used as an alternative to conventional smears for assessing

breast lesions. The study population was comprised of the subjects visiting the Outpatient department of the Institute with the complaint of breast lesions.

The present study included a total of 374 female subjects with breast FNAC obtained from the palpable breast lesions. After the final inclusion of the study subjects, detailed history was recorded for all the subjects followed by the clinical examination. After explaining the detailed study design, informed consent was taken from all the study subjects.

Under the strict aseptic and sterile condition, fine-needle aspiration was done for the breast lump with a 23-gauge needle. For each subject, two passes were done where the first pass was used for the smear preparation conventionally. Under toluidine preparation, adequacy was evaluated on the site for one air-dried smear. 95% ethanol fixation was done for one wet smear immediately for 30 minutes at least, followed by staining with Papanicolaou (Pap) stain. The remaining slides were stained using May-Grunwald-Giemsa (MGG) stain.

Concerning LBC, a second pass was given and aspirated where aspirate was rinsed in 5 to 7ml tube having CytoRich preservative fluid followed by evaluation in the laboratory. Before processing, the sample was kept for fixation for a minimum of 1 hour followed by centrifugation for 10 minutes at 600g, and the supernatant was decanted. Also, in the centrifuge tube, Tri's buffer was added, vortexed for 25 s,followed by centrifugation for 5 minutes.

Various criteria were used for the comparison of LBC smear and conventional smear. These included: Background blood and cell-debris, Informative background (such as stromal fragments, bare nuclei in benign cases, and tumor diathesis in malignant cases), Cytomorphological details including the presence of cells in monolayer, nuclear details (including nuclear size, membrane irregularity, chromatin pattern, and visibility of nucleoli), and cytoplasmic details (including cytoplasmic borders, vacuolization, granularity, presence of pigment, etc.), Cellular architecture including the presence of cell clusters, branching sheets, papillary fragments, etc., and cellularity.

For each feature, the scoring was done individually. The collected data were subjected to the statistical evaluation using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation. The data were expressed in percentage and number, and mean and standard deviation. The level of significance was kept at $p<0.05$.

## RESULTS

The present prospective clinical study was conducted to comparatively evaluate the utility and efficacy of Liquid-Based Cytology over conventional smears for assessment of the breast lesion, and to assess their use as an alternative to the conventional preparation. The present study included a total of 374 female subjects with breast FNAC obtained from the palpable breast lesions. For the case distribution based on the nature of the lesion in the study subjects, it was seen that indeterminate/inconclusive results on LBC were seen in 4.01% (n=15) subjects and on conventional smear in 5.08% (n=19) subjects. Malignant lesion on LBC were seen in 40.90% (n=153) subjects and on conventional smear in 37.16% (n=139) subjects. Atypical findings suggesting malignancy were seen in no subject on LBC and conventional

smear in 3.47% (n=13) subjects. Atypical findings suggesting benign state was seen on LBC and conventional smear in 8.02% (n=30) subjects and 5.08% (n=19) subjects respectively. Benign lesion on LBC and conventional smear were seen in 47.05% (n=176) and 49.19% (n=184) subjects respectively as shown in Table 1.

For the comparison of LBC and conventional smear for assessing breast lesion in the study subjects, inadequate results for LBC and conventional smear were seen in 4.01% (n=15) and 5.08% (n=19) subjects respectively. Carcinoma was seen on LBC and conventional smear in 40.90% (n=153) and 37.16% (n=139) subjects, atypical findings suggesting malignancy were seen in in no subject on LBC and conventional smear in 3.47% (n=13) subjects, whereas, atypical findings suggesting benign lesion were seen in 8.02% (n=30) subjects and 5.08% (n=19) subjects respectively. Gynecomastia and fat necrosis were seen on LBC and conventional smear in 2.94% (n=11) subjects each. Galactocele was seen on LBC and conventional smear in 1.06% (n=4) subjects respectively. The inflammatory lesion was seen on LBC and conventional smear in 14.97% (n=56) subjects each and fibrocystic disease in 1.06% (n=4) subjects on both liquid-based cytology and conventional smear. Fibroadenoma was seen in 24.06% (n=90) subjects using LBC and 26.20% (n=98) subjects on conventional smear (Table 2).

Concerning the assessment of the significance of cytologic features using LBC and conventional smear in the study subjects, background blood-debris (LBC) and blood debris (conventional smear) had a z-score of -7.246 with better results with background blood debris (Figure 2) which was statistically significant with $p<0.001$. Similar results were seen for the informative background (Figure 3) where LBC showed significantly better results with respective z and p-value of -6.254 and <0.001 respectively. Cytoplasmic detail showed no significant difference between LBC and conventional smear with $p=0.975$. Nuclear details (Figure 4) had a statistically non-significant difference on LBC and conventional smear with $p=0.436$. A similar non-significant difference was seen between LBC and conventional smear for monolayer, cytoarchitectural pattern, and cellularity (Figure 1) with respective p-values of 0.469, 0.117, and 0.672 respectively as shown in Table 3.

## DISCUSSION

The present prospective clinical study was conducted to comparatively evaluate the utility and efficacy of Liquid-Based Cytology over conventional smears for assessment of the breast lesion, and to assess their use as an alternative to the conventional preparation. The present study included a total of 374 female subjects with breast FNAC obtained from the palpable breast lesions. For the case distribution based on the nature of the lesion in the study subjects, it was seen that indeterminate/inconclusive results on LBC were seen in 4.01% (n=15) subjects and on conventional smear in 5.08% (n=19) subjects. Malignant lesion on LBC were seen in 40.90% (n=153) subjects and on conventional smear in 37.16% (n=139) subjects. Atypical findings suggesting malignancy were seen in no subject on LBC and conventional smear in 3.47% (n=13) subjects. Atypical findings suggesting benign state was seen on LBC and conventional smear in 8.02% (n=30) subjects and 5.08% (n=19) subjects respectively. Benign lesion on LBC and conventional smear were seen in 47.05% (n=176) and 49.19% (n=184) subjects respectively. These findings were consistent with the results of Singh P et

al[5] in 2016 and Ryu HS et al[6] in 2013 where authors showed similar case distribution based on the nature of the lesion as the present study.

Concerning the comparison of LBC and conventional smear for assessing breast lesion in the study subjects, inadequate results for LBC and conventional smear were seen in 4.01% (n=15) and 5.08% (n=19) subjects respectively. Carcinoma was seen on LBC and conventional smear in 40.90% (n=153) and 37.16% (n=139) subjects, atypical findings suggesting malignancy were seen in in no subject on LBC and conventional smear in 3.47% (n=13) subjects, whereas, atypical findings suggesting benign lesion were seen in 8.02% (n=30) subjects and 5.08% (n=19) subjects respectively. Gynecomastia and fat necrosis were seen on LBC and conventional smear in 2.94% (n=11) subjects each. Galactocele was seen on LBC and conventional smear in 1.06% (n=4) subjects respectively. The inflammatory lesion was seen on LBC and conventional smear in 14.97% (n=56) subjects each and fibrocystic disease in 1.06% (n=4) subjects on both liquid-based cytology and conventional smear. Fibroadenoma was seen in 24.06% (n=90) subjects using LBC and 26.20% (n=98) subjects on conventional smear. These results were in agreement with the studies of Kumar N et al[7] in 2011 and Liew PL et al[8] in 2011 where a similar comparison was seen for LBC and conventional smear for assessing breast lesion.

For assessing the significance of cytologic features using LBC and conventional smear in the study subjects, background blood-debris (LBC) and blood debris (conventional smear) had a z-score of -7.246 with better results with background blood debris which was statistically significant with $p<0.001$. Similar results were seen for the informative background where LBC showed significantly better results with respective z and p-value of -6.254 and <0.001 respectively. Cytoplasmic detail showed no significant difference between LBC and conventional smear with $p=0.975$. Nuclear details had a statistically non-significant difference on LBC and conventional smear with $p=0.436$. A similar non-significant difference was seen between LBC and conventional smear for monolayer, cytoarchitectural pattern, and cellularity with respective p-values of 0.469, 0.117, and 0.672 respectively. These findings were comparable to the results of Tripathy K et al[9] in 2015 and Muddegowda PH et al[10] in 2011where authors showed better and significant results with LBC for background and blood debris compared to conventional smear.

## CONCLUSION

Within its limitations, the present study concludes that LBC can be a promising tool for cytology field is liquid-based cytology with the potential to reduce slides number screened for each case and also reduce the turn-around-time. However, the present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample size and longer monitoring period will help reach a definitive conclusion.

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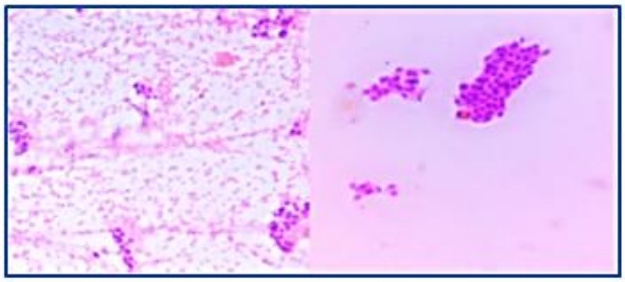
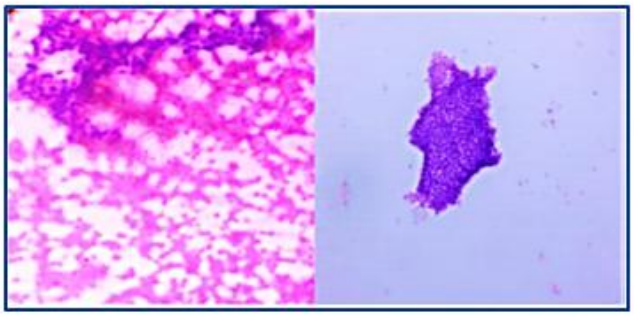
**ATLAS**



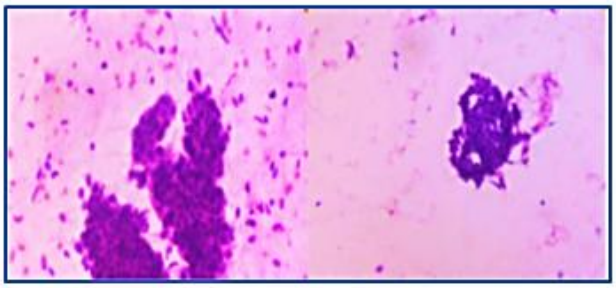
a) CS b) LBC

**Figure 1: Cellularity comparison in the study subjects**



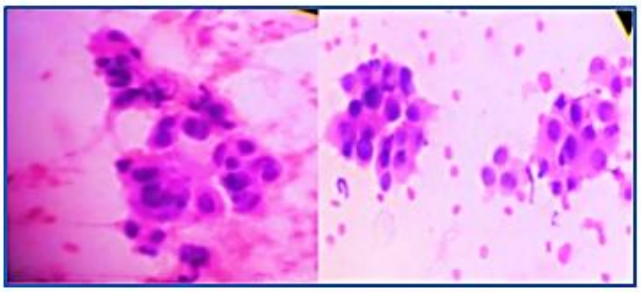
a) CS b) LBC

**Figure 2: Background comparison in the study specimens**



a) CS b) LBC

**Figure 3: Informative background comparison in the study subjects**



a) CS b) LBC

**Figure 4: Comparison of nuclear and cytoplasmic details in the study subjects**

## TABLES

| Lesion | LBC (Liquid-based cytology) | | Conventional Cytology | |
|---|---|---|---|---|
| | % | n | % | n |

| | | | | |
|---|---|---|---|---|
| **Indeterminate/inconclusive** | 4.01 | 15 | 5.08 | 19 |
| **Malignant** | 40.90 | 153 | 37.16 | 139 |
| **Atypical pointing malignancy** | - | - | 3.47 | 13 |
| **Atypical pointing benign state** | 8.02 | 30 | 5.08 | 19 |
| **Benign** | 47.05 | 176 | 49.19 | 184 |
| **Total** | 100 | 374 | 100 | 374 |

**Table 1: Case distribution based on nature of the lesion in the study subjects**

| **Lesion** | **LBC (Liquid-based cytology)** | | **Conventional Cytology** | |
|---|---|---|---|---|
| | **%** | **N** | **%** | **N** |
| **Inadequate** | 4.01 | 15 | 5.08 | 19 |
| **Carcinoma** | 40.90 | 153 | 37.16 | 139 |
| **Atypical pointing malignancy** | - | - | 3.47 | 13 |
| **Atypical pointing benign state** | 8.02 | 30 | 5.08 | 19 |
| **Gynaecomastia** | 2.94 | 11 | 2.94 | 11 |
| **Fat necrosis** | 2.94 | 11 | 2.94 | 11 |
| **Galactocele** | 1.06 | 4 | 1.06 | 4 |
| **Inflammatory lesion** | 14.97 | 56 | 14.97 | 56 |
| **Fibrocystic disease** | 1.06 | 4 | 1.06 | 4 |
| **Fibroadenoma** | 24.06 | 90 | 26.20 | 98 |
| **Total** | 100 | 374 | 100 | 374 |

**Table 2: Comparison of LBC and conventional smear for assessing breast lesion in the study subjects**

| **Cytologic features** | **z-scores** | **p-value** |
|---|---|---|
| **Background blood-debris (LBC) and blood debris (conventional smear)** | -7.246 | **<0.001** |
| **Informative background LBC and Informative background conventional smear** | -6.254 | **<0.001** |
| **Cytoplasmic details LBC and conventional smear** | -0.036 | 0.975 |
| **Nuclear details LBC and conventional smear** | -0.7777 | 0.436 |
| **Monolayer LBC and conventional smear** | -0.726 | 0.469 |
| **Cyto-architectural pattern LBC and conventional smear** | -1.577 | 0.117 |
| **Cellularity LBC and conventional smear** | -0.428 | 0.672 |

**Table 3: Assessing the significance of cytologic features using LBC and conventional smear in the study subjects**