Comparative Analysis of Liquid based and Conventional Cytology Smears in Fine Needle Aspirates from Breast Lesions

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Abstract

Context: Liquid-based cytology (LBC) is being extensively used for the evaluation of both gynecological and non-gynecological specimens. Suspension of cells in monolayer makes better morphological assessment possible. Along with this, inherent morphological changes such as altered, reduced, or lost background material, fragmented cell clusters, smaller cell size, nucleolar prominence, etc., need to be considered.

Aim: Present study was aimed at comparative evaluation of utility of LBC versus conventional smear (CS) in assessing breast lesions and whether it can be used as an alternative to conventional preparation.

Settings and Design: Present study was a prospective study in which 75 cases of breast fine-needle aspiration cytology from patients with palpable breast lumps constituted the study group.

Material and Methods: The first pass was used for CS and LBC; a second pass was given. The representative CS and LBC smears were compared using several criteria.

Statistics: Each feature was scored individually and evaluated statistically using Wilcoxon’s signed rank test on the SPSS program.

Results: A statistically significant difference was found in informative background and background blood-debris, whereas the difference was not statistically significant in other features such as cellularity, cytoarchitectural pattern, presence of monolayer, and nuclear and cytoplasmic details.

Conclusion: LBC is a promising technique in the field of cytology. It has the potential to decrease the number of slides screened per case and decrease the turn-around-time.

Keywords: Conventional smear, fine-needle aspiration cytology, liquid-based cytology.

INTRODUCTION

With growing awareness in the general population, a lady with a breast lump is one of the most common presentations in outpatient department. Diseases of breast usually present as palpable lumps, nipple discharge, or abnormalities on imaging studies.[1]

Cytology can explore breast lesions in distinct ways and has a vital role in both screening and diagnostic purposes. Fine-needle aspiration cytology (FNAC) of breast lump is a quasi-routine clinical procedure and is an important part of triple assessment of palpable breast lumps. It is an accurate, rapid, easy to perform, and cost-effective procedure for evaluating breast lesions.[2]

Conventional smears (CSs), though useful in diagnosis, are tedious and time-consuming to screen because of the non-uniform slide preparation and fixation. Features usually associated with CS such as thick, overlapping cellular areas, obscuring inflammation, blood, and air drying artifacts result in poor nuclear and cellular preservation.[3]

Liquid-based cytology (LBC) is being extensively used for the evaluation of both gynecological and non-gynecological specimens. In this technique, cells are rinsed into a liquid preservative medium, and instead of being smeared, samples are processed on automated devices. This enables cells to be suspended in a monolayer making better morphological assessment possible.[4,5] Along with this, a number of inherent morphologic changes need to be considered that include altered, reduced, or lost background material, fragmented cell clusters, smaller cell size, well preserved nuclear details, prominent nucleoli, and easily visualized cytoplasm. Other advantages of LBC include rapid fixation and even distribution of cells over a smaller slide area.[6,7]

Thus, the time required for evaluation by screeners and cytopathologists is reduced. Moreover, the residual material

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in fixative solution can be used for ancillary studies such as immunocytochemistry.\(^{(4)}\) Keeping in view the frequency of breast lesions and their potential curability, present study was aimed at comparative evaluation of utility of LBC versus CS in assessing breast lesions and whether it can be used as an alternative to conventional preparation.

**Materials and Methods**

Present study was a prospective study in which 75 cases of breast FNAC from patients with palpable breast lumps constituted the study group. After detailed history, clinical examination and informed consent of patients, FNA of breast lump was performed under aseptic conditions using sterile disposable 23 gauge needles and 20 cc syringes. In each case, two passes were performed. The first pass was used for conventional preparation. One air-dried smear was subjected to adequacy evaluation under toluidine preparation on the site. One wet smear was immediately fixed in 95% ethanol for a minimum of 30 min and later on, was stained with Papanicolaou (Pap) stain. Rest of the slides were stained with May-Grunwald-Giemsa (MGG) stain in the laboratory. For LBC, a second pass was given and the aspirate was rinsed in a tube containing 5 to 7 ml of CytoRich preservative fluid and was transported to the laboratory. The sample was kept for a minimum of 1 h before processing to allow for adequate fixation. The sample was centrifuged at 600 g for 10 min and supernatant was decanted. In addition, 4 to 6 ml of Tris buffer was added to the centrifuge tube, vortexed for 25 s, and the tubes were then centrifuged for 5 min. The supernatant was decanted, and further, vortexing for 25 s was done. The labeled centrifuge tube holders were loaded onto the PrepStain slide processor for processing, which included slide preparation as well as staining. From each case, one slide was prepared that was auto-stained with Pap stain in the slide processor.

The representative conventional and LBC smears were compared using several criteria [Table 1]. The representative CS and LBC smears were compared for:

- **Cellularity**
- **Cellular architecture** including presence of cell clusters, branching sheets, papillary fragments, etc.
- **Cytomorphological details** including 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.).
- **Informative background** (such as stromal fragments, bare nuclei in benign cases, and tumor diathesis in malignant cases)
- **Background blood and cell-debris**.

Each feature was scored individually and evaluated statistically using Wilcoxon’s signed rank test on the SPSS program. Cytological diagnosis in each case was recorded and tabulated to yield \(P\) value. The \(P\) value <0.001 was considered significant, and the \(P\) value <0.005 was considered highly significant.

### Results

The present study included 75 cases of breast lesions that comprised of 69 females and 6 males. The observations are seen in Tables 2-4. All the cases were categorized into benign, atypical favoring benign, atypical favoring malignant, malignant, and inconclusive/indeterminate on both CS and LBC [Table 2]. On conventional cytology, 37 cases were diagnosed as benign, four as atypical favoring benign, two as atypical favoring malignant, 28 as malignant, and four cases were inconclusive/indeterminate for opinion, whereas on LBC, 35 cases were diagnosed as benign, six cases as atypical favoring benign, 31 as malignant, and three cases were inconclusive/indeterminate for opinion. Two cases diagnosed as atypical favoring benign on LBC were diagnosed as fibroadenoma on conventional cytology that were confirmed as fibroadenoma on histopathology [Table 3] This difference could be attributed to loss of informative background such as stromal fragments, bare nuclei, etc., on LBC. Twenty-eight cases of breast carcinoma were adequately diagnosed on CS

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

Cellularity was assessed and graded as follows:

- **Zero** - No duct epithelial cells seen.
- **Scant** - Few groups of duct epithelial cells seen per high-power field.
- **Adequate** - Numerous groups of epithelial cells, each group comprising of 8 to 10 ductal epithelial cells.
- **Abundant** - Numerous groups, clusters, and sheets of ductal epithelial cells with presence of bare nuclei and stromal fragments in the background.
Table 2: Distribution of cases as per nature of lesion (n=75)

| Nature of lesion | Conventional cytology | Liquid-based cytology |
|------------------|-----------------------|-----------------------|
| Benign           | 37                    | 35                    |
| Atypical favoring benign | 04                   | 06                    |
| Atypical favoring malignant | 02 | -                     |
| Malignant        | 28                    | 31                    |
| Inconclusive/indeterminate | 04 | 03                   |
| Total            | 75                    | 75                    |

Table 3: Distribution of cases and comparison of various lesions diagnosed on conventional and liquid-based cytology (n=75)

| Type of lesion          | Conventional cytology | Liquid-based cytology |
|-------------------------|-----------------------|-----------------------|
| Fibroadenoma            | 20                    | 18*                   |
| Fibrocystic disease     | 01                    | 01                    |
| Inflammatory lesion     | 11                    | 11                    |
| Galactocele             | 01                    | 01                    |
| Fat necrosis            | 02                    | 02                    |
| Gynecomastia            | 02                    | 02                    |
| Atypical favoring benign | 04                   | 06                    |
| Atypical favoring malignant | 02           | -                     |
| Carcinoma               | 28                    | 31*                   |
| Inadequate              | 04                    | 03                    |
| Total                   | 75                    | 75                    |

*18 cases of fibroadenoma were adequately diagnosed on CS as well as LBC [Figure 1]. In addition, two cases diagnosed as fibroadenoma on CS were diagnosed as atypical favoring benign on LBC due to lack of informative background.

28 cases of carcinoma were adequately diagnosed on CS as well as LBC [Figure 2]. In addition, 02 cases diagnosed as proliferative mammary lesion with atypia were diagnosed as carcinoma on LBC. 01 case diagnosed as inadequate/inconclusive on CS was also diagnosed as carcinoma on LBC.

As far as cellularity was concerned, we found no statistically significant difference between LBC and conventional cytology (P value = 0.670) similar to Leung et al.,[11] Dey et al,[12] and Veneti et al.[9] Biscotti et al.[8], however, reported that cellularity was higher in samples processed by LBC (P value = 0.05). The difference may be because of the nature of lesions sampled and variation in the pricks given. However, one of the studies conducted by Perez et al.[13] reported a lower cellularity in LBC. They concluded that this could have been affected by the split sampling technique used for obtaining LBC smears.

On the basis of cytoarchitectural pattern, there was no statistically significant difference found in our study, similar to studies conducted in the past.[8-12,14] In addition, we found that cellular aggregates were more fragmented, shortened, and less distinct. Similar findings were observed by Michael et al.[15] though the difference was not statistically significant. Ryu et al.[14] also reported that when compared with conventional preparation, SurePath produced prominent three-dimensional

Table 4: Statistical analysis of the various cytological features assessed by conventional and liquid-based cytology (Wilcoxon signed rank test)

| Cytological features                        | Z-score | Asymptomatic significance (2 tailed) |
|---------------------------------------------|---------|-------------------------------------|
| Cellularity LBC-                           | −0.426  | 0.670                               |
| Cellularity CS                             | −1.575  | 0.115                               |
| Cytoarchitectural pattern LBC-              | −0.728  | 0.467                               |
| Cytoarchitectural pattern CS                | −0.775  | 0.438                               |
| Monolayer LBC-                             | −0.757  | 0.499                               |
| Monolayer CS                               | −0.034  | 0.973                               |
| Nuclear details LBC-                       | −6.252  | <0.001*                             |
| Nuclear details CS                         | −7.248  | <0.001*                             |
| Cytoplasmic details LBC-                   | −0.775  | 0.438                               |
| Cytoplasmic details CS                     | −0.728  | 0.467                               |
| Informative background LBC                 | −0.775  | 0.438                               |
| Informative background CS                  | −6.252  | <0.001*                             |
| Background blood-debris LBC                | −7.248  | <0.001*                             |
| Background blood-debris CS                 | −0.001* |                                     |

*Statistically significant

Discussion

LBC is generally favored over conventional cytology preparations for the evaluation of gynecological specimens. However, studies comparing the diagnostic accuracy of LBC and CS in the evaluation of non-gynecological specimens such as breast cytology have drawn variable results.

In our study, two separate pricks were given, one for CS and the aspirate from the second prick was used for LBC similar to Mygdakos et al.,[4] Biscotti et al.[8] Veneti et al.[9] and Tripathy et al.[10]

samples processed with LBC technique (P value < 0.001). Marked reduction in the background blood-debris was seen in smears prepared by LBC as compared to conventional cytology (P value < 0.001).
Sharma, et al.: Comparison of LBC and conventional smears in breast lesions

Figure 1: (a) Conventional smear of fibroadenoma showing stromal fragment in fibroadenoma along with sheets of benign ductal epithelial cells (MGG stain x100). (b) Fibroadenoma on LBC (Papanicolaou stain x200). (c) LBC smear of fibroadenoma showing sheets of benign ductal epithelial cells along with a large stromal fragment (Papanicolaou stain x400). (d) Showing LBC smear of fibroadenoma in which myoepithelial cells are seen in a different plane of focus (Papanicolaou stain x400)

configuration for epithelial clusters that caused difficulty in recognizing nuclear characteristics.

We observed that the presence of three-dimensional clusters were more in LBC compared to conventional cytology similar to Ryu et al. In previous studies, smears prepared by LBC have been reported better than CS on the basis of presence of monolayer. In the present study, we observed no statistically significant difference between the smears prepared by the two techniques. One study conducted on lymph node aspirates by Singh et al. also found no statistically significant difference regarding the presence of monolayers similar to present study. This difference may be attributed to the type of preparation used (ThinPrep versus SurePath) and need to be studied further.

Similar to Perez et al., we observed that nuclear details were attenuated on LBC as compared to conventional preparation and the size was diminished, though the difference in our study was not statistically significant. However, Dey et al., Michael et al., Gerhard et al., and Tripathy et al. all observed better nuclear details in LBC smears than in conventional preparation.

Cytoplasmic details found in our study were similar to those observed by Veneti et al., and the difference was not statistically significant. However, Dey et al., Michael et al., and Mygdakos et al. observed better cytoplasmic details in LBC than in CSs.

In the present study, informative background was retained in some, but lost in few LBC preparations as compared to conventional preparation. Consequently, atypical favoring benign lesions on LBC were adequately diagnosed as fibroadenoma on CS. This difference because of the loss of informative background was statistically significant. Veneti et al. stated that there was a lack of informative background.

Michael et al., Perez et al., and Tripathy et al. noted that there was a loss of stromal fragments in smears prepared from LBC.

Hoda stated that informative background was reduced or altered in liquid-based preparation. He reviewed that extracellular elements appeared as small, dense globules, thread like, or fibrinous material with loss of tumor diathesis, similar to Leung et al. There was lesser amount of stroma with fewer bipolar cells and loss of epithelial-stromal relationship. Mygdakos et al. found informative background as good as CSs in their study.

On the basis of background blood-debris, we found a statistically significant difference between LBC and conventional cytology. We observed that there was a significant loss of background obscuring elements in smears prepared from LBC (P value < 0.001).

LBC is generally favored over conventional preparation for evaluation of gynecological cytology specimens. However, studies comparing the diagnostic accuracy and morphology of these preparations have shown variable results in the evaluation of non-gynecological cytological specimens. Accuracy is the ultimate diagnostic goal of FNAC with secondary goals to achieve as much safety, speed, and cost-effectiveness as possible. The cytopreparatory technique used to prepare the specimen is integral to obtaining diagnostic accuracy. Regarding specimen quality, LBC are less time consuming to screen and easier to interpret, as the cells are limited to a smaller area on a cleaner background with appropriate cellular preservation. However, fixation artifacts, cell shrinkage, loss of nuclear details, and informative background, as observed in the present study are significant problem areas for use of LBC as the sole technique for FNAC. Further, the LBC preparation is expensive than conventional preparation and requires some experience for interpretation. Hence, it can be concluded that LBC can supplement but cannot replace conventional cytology. Moreover, studies with more number and wide spectrum of breast lesions are required if LBC is the first and the only methodology applied.

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Conflicts of interest
There are no conflicts of interest.
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