Manual Liquid Based Cytology in Breast Fine Needle Aspiration – Comparison with the Conventional Smear

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Abstract

**Introduction:** Breast carcinoma is the malignant tumour that causes highest mortality among women worldwide. Liquid based smears are superior to conventional smears (CS) with regard to monolayer cell preparation, cell preservation and clear background. Liquid based cytology (LBC) has gained acceptance for use in prospective studies, because of its usefulness in immunocytochemistry (ICC) and molecular biology.

**Objectives:** To study the performance of Manual liquid based cytology (MLBC) of neoplastic breast lesions in comparison to conventional fine needle aspiration (FNA) smears and to correlate the findings with Histopathology wherever possible.

**Results/Observations:** Smears for MLBC and conventional FNA were prepared from the samples from 100 female patients in the age group of 15-70 years. Simultaneously unstained smears were also prepared to be utilized for ICC. Sensitivity and specificity for both MLBC and CS was 95.2% and 100% respectively. Positive predictive value and negative predictive value for MLBC and CS was 100% and 96.9% respectively. Estrogen receptor analysis performed on five unstained MLBC smears as well on biopsy sections of the same five cases were reported as negative.

**Conclusion:** Liquid based cytology can be safely used as an important adjunct to conventional FNA. Liquid based cytology ensures better cellular preservation, less cell overlapping and elimination of blood and excessive inflammation compared to CS. Manual liquid cytology is a technique which can be as effective a diagnostic tool as CS in low-resource settings like India. Utilization for ancillary tests such as ICC for hormonal receptors and molecular biology is an additional advantage of MLBC.

**Keywords:** Breast, MLBC, Immunocytochemistry

I. Introduction

With more than 10,00,000 cases occurring worldwide annually, breast carcinoma is the most common malignant tumour causing mortality. In our country as well, the incidence of breast carcinoma is steadily on the rise. Fine Needle Aspiration Cytology (FNAC) of breast lumps is considered an important mode of diagnosing breast neoplasms although histopathological diagnosis is a widely accepted confirmatory mode of diagnosis. Currently, Fine needle aspiration of the breast is hindered by factors such as low cellularity, poor preservation and obscuring background hence there is a definite need for utilization of Liquid Based Preparations (LBP) which have recently become popular. Fine needle aspiration of the breast is hindered by factors such as low cellularity, poor preservation and obscuring background. It is easier and less time consuming to screen and interpret liquid based smears because the cells are limited to smaller areas on clear backgrounds with excellent cellular preservation. The screening time of the cytopathologist is reduced but the most important advantage is the availability of the residual sample which can be used for additional ancillary techniques, such as Immunocytochemistry (ICC), flow cytometry and molecular biology. In fact, the LBP method allows variable amount of cells to be stored. The cost of most commercially available LBC systems is extremely expensive and the problem is further compounded by the need for trained individuals and periodic maintenance of the equipments, this is not viable for resource-limited settings. Many workers like Alves et al\(^1\) and Lee et al\(^2\) have devised an improved manual method for preparation of LBC smears. The present study aimed to explore the role of Manual liquid based cytology (MLBC) in breast FNA and to compare it with Conventional Smear (CS) by using a cost effective manual liquid based method for a resource limited setting like ours.
II. Methodology

The present study was undertaken to prepare breast FNA smears using the manual method of liquid-based cytology (MLBC) and compare it with the conventional smears, and correlate with histopathological findings wherever possible. Samples were collected from 100 patients in the age group of 15 to 70 years from July 2013 to July 2015. Fine needle aspiration of the breast was performed using a 22-gauge needle and in each case three passes were performed, two for the conventional method and one for MLBC. From the two passes for conventional method 4-6 smears were prepared; two of these smears were air-dried for staining with May-Grunwald Giemsa (MGG) while the rest of the smears were used for Papanicolaou (Pap) and Haematoxylin & Eosin (H&E) staining. The aspirate material collected in the third pass was utilized for MLBC. The aspirate material was immediately put into a vial containing the fixative solution. The fixative that was used for MLBC consisted of equal amounts of propanol and glacial acetic acid. The sample was collected and mixed with equal parts of fixative. It was centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and excess fixative was blotted. One to two ml of polymer solution (containing 2 gm of agarose, 10 ml of polyethylene glycol, 2 ml of poly-L-lysine and 88 ml of distilled water) was added to the deposit. It was again centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted then 1-2 ml of phosphate buffer was added to the deposit. It was again centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and the deposit mixed properly. The deposit was then pipetted in a circular motion on to a glass slide. The slides were dried after which they were stained with Pap stain. Simultaneously aspirate collected for conventional FNA was evenly spread onto a glass slide and immediately fixed in alcohol fixative (95% propanol). After fixation, smears were stained and unstained smears were also prepared from the aspirates in order to be utilised for ICC wherever possible. The cases in which mastectomy, lumpectomy and core biopsy was done, specimens were collected in 10% formalin and allowed to fix overnight. Detailed gross examination was done and bits were given. Paraffin embedded H&E stained sections were obtained and studied under light microscopy.

In order to standardize the present study the following scoring system was utilized:

<table>
<thead>
<tr>
<th>Cytologic features</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Nil</td>
<td>Scanty</td>
<td>Adequate</td>
<td>Abundant</td>
</tr>
<tr>
<td>Background blood, cell debris</td>
<td>Nil</td>
<td>Occasional</td>
<td>Good amount</td>
<td>Abundant</td>
</tr>
<tr>
<td>Informative background</td>
<td>Absent</td>
<td>Present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monolayer</td>
<td>Absent</td>
<td>Occasional</td>
<td>Many monolayer cells</td>
<td></td>
</tr>
<tr>
<td>Cell architecture</td>
<td>Not recognized</td>
<td>Partially recognized</td>
<td>Well recognized</td>
<td></td>
</tr>
<tr>
<td>Nuclear detail</td>
<td>Poor</td>
<td>Fair</td>
<td>Good</td>
<td>Very good</td>
</tr>
<tr>
<td>Cytoplasmic detail</td>
<td>Poor</td>
<td>Fair</td>
<td>Good</td>
<td>Very good</td>
</tr>
</tbody>
</table>

III. Statistical Methods

Cytological diagnosis was correlated with histopathology and the efficacy of cytology was estimated by using the methodology of Galen and Gambino as follows:-

\[
\text{Sensitivity} = \frac{TP}{TP + FN} \times 100 \\
\text{Specificity} = \frac{TN}{TN + FP} \times 100 \\
\text{Positive Predictive Value} = \frac{TP}{TP + FP} \times 100 \\
\text{Negative Predictive Value} = \frac{TN}{TN + FN} \times 100
\]

TP = True Positive  FP = False positive
TN = True Negative  FN = False Negative

IV. Results

The present study was undertaken to compare the cytological features of MLBC smears of neoplastic breast lesions with conventional FNA smears and correlate with histopathological findings in order to determine its diagnostic accuracy. Age group of patients ranged from 15 to 70 years with a mean age of 35.55 years. 53% of tumours were located in the right breast whereas 47% of tumours were located in the left breast. In the present study 100 smears prepared by MLBC were studied, out of which 66 cases (66.0%) were reported as benign and 34 cases (34.0%) were reported as malignant. Similarly out of the 100 smears prepared by conventional method, 66 cases (66.0%) were reported as benign and 34 cases (34.0%) were reported as malignant.
benign and 34 cases (34.0%) were reported as malignant (Table 2). Among the 100 cases, attempt at Cytohistopathological correlation was possible in only 52 cases (52.0%) as we received biopsy specimens of only 52 cases whereas we did not receive the biopsy specimens of the remaining 48 cases (48.0%). Out of the 52 cases in which Cytohistopathological correlation was done, 31 were reported as benign whereas the remaining 21 cases were reported as malignant. On the conventional FNA smears and the MLBC smears the most common lesion was Fibroadenoma (Fig – 1,2). On Histopathological examination (HPE), the most common benign lesion reported was Fibroadenoma (Fig – 3). The most common malignant lesion reported was Invasive ductal carcinoma not otherwise specified (NOS) type (Fig – 6). In the present study we found that the MLBC method was comparable to the CS, therefore the diagnostic accuracy of MLBC was similar to CS. Histopathological Correlation was possible in 52 out of the 100 cases. Out of the 52 cases, 31 reported as benign on CS/MLBC were confirmed as benign on HPE whereas 1 case which was reported as benign on CS and MLBC turned out to be malignant on HPE. The remaining 20 cases were reported as malignant on CS and MLBC and turned out to be malignant on HPE (Table 3). Manual liquid based cytology and conventional smears are equally sensitive and specific in diagnosis of malignant lesions. Estrogen receptor (ER) analysis was performed on 5 unstained MLBC smears as well on biopsy sections of the same 5 cases. On both MLBC smears and biopsy sections, ER was reported as negative.

Fig 1 CS (H&E, x100) – Fibroadenoma

Fig 2 MLBC (Pap, x200) – Fibroadenoma

Fig 3 HPE (H&E, x40) – Fibroadenoma
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Figure 6: HPE (H&E, x200) – Infiltrating Duct Carcinoma NOS type

Table 2: Distribution of cases into benign and malignant on Manual liquid based cytology and Conventional smear

<table>
<thead>
<tr>
<th>Lesions</th>
<th>No. of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>66</td>
<td>66.0</td>
</tr>
<tr>
<td>Malignant</td>
<td>34</td>
<td>34.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3: Distribution of cases into benign and malignant on Histopathology

<table>
<thead>
<tr>
<th>Lesions</th>
<th>No. of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>51</td>
<td>59.6</td>
</tr>
<tr>
<td>Malignant</td>
<td>21</td>
<td>40.4</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>100.0</td>
</tr>
</tbody>
</table>

V. Discussion

Currently, breast FNA has a significant role in the evaluation of breast lumps however the success of breast FNA depends immensely upon correct preparation of cytological smears and skills of the person performing the procedure which can ultimately lead to diagnostic failure. Liquid based smears are better than the conventional cytological preparations since they avoid limiting factors such as obscuring material, air-dried artifacts and irregular thickness of the smears. In addition LBC eliminates the obscuring elements seen in the conventional smears and also provides superior cell preservation. On the other hand, alterations in architecture and cellular morphology as well as loss of informative background are usually seen in FNA smears prepared by LBC. In the present study, the age range and mean age was comparable with other studies. In our study 53% of tumours were located in the right breast whereas 47% of tumours were located in the left breast which was similar to the findings of Ryu HS et al. In the present study we compared 100 MLBC and conventional FNA smears on following Cytomorphological parameters:

Cellularity

In the present study the cellularity of MLBC smears was initially inferior to CS but after switching from split sampling technique to utilization of a separate pass entirely for MLBC the cellularity of MLBC smears became almost equivalent to CS as was reported by Dey P et al (Table 4). Perez-Reyes et al employed split sampling technique where they divided the aspirate into two halves, one for LBC and the other for CS, hence in their study the cellularity of LBC was inferior to CS. Gerhard R et al observed that the number of passes performed and the skills of the person performing the procedure determine the quality and cellularity of the samples.

Architectural Features

In the present study there was less nuclear overlapping of cells and more fragmented and dis cohesive clusters of cells, this resulted in better assessment of cell morphology and architecture (Table 4). The aforementioned findings were also observed by various other authors. But the presence of small clusters and loss of cohesion may lead to an erroneous diagnosis of malignancy as observed by Gerhard R et al.

Cell Changes

In the present study the cytoplasmic details of cells on MLBC were similar to the cells on CS (Table 4). Most of the smears showed better-preserved cells with enhanced nuclear detail, including a more pronounced nucleoli which was also observed by various other authors. Thus Micheal CW et al, Biscotti CV et al and Feoli F et al observed that in order to avoid diagnostic errors it is advisable to undergo prior training in

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LBC to understand the morphological changes produced by LBC. Most of the MLBC smears showed cells arranged in monolayered sheets that enabled us to study the cell morphology even better which was also observed by Pawar PS et al. In the present study that there was an apparent decrease in number of myoepithelial cells in MLBC smears as compared to CS which was also observed by other authors.  

### Background Elements

Most of the MLBC smears in the present study had a clean background as a result of the elimination of obscuring elements such as blood, excessive inflammation and cellular debris which was also observed in other studies. We faced the problem of background blood among the first batch of MLBC slides but were able to correct it by modifying the fixative used which comprised of equal amounts of propanol and glacial acetic acid. On the other hand the presence of a clean background in the MLBC smears meant that there was also reduction and in some cases even absence of informative background such as stromal fragments (Table 4). This finding was also described in other studies. 

<table>
<thead>
<tr>
<th>Morphological Features</th>
<th>Manual liquid based cytology</th>
<th>Conventional smears</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Initially slightly inferior than CS but later comparable to CS</td>
<td>Equal and in some cases slightly superior than MLBC</td>
</tr>
<tr>
<td>Clean Background</td>
<td>Present in most cases</td>
<td>Absent and obscuring factors noted</td>
</tr>
<tr>
<td>Informative background</td>
<td>Usually absent</td>
<td>Present</td>
</tr>
<tr>
<td>Monolayer</td>
<td>Monolayer in most of the cases</td>
<td>Less monolayering with more overlapping cells</td>
</tr>
<tr>
<td>Cell architecture</td>
<td>Slightly superior to CS</td>
<td>Slightly inferior to MLBC</td>
</tr>
<tr>
<td>Cytoplasmic detail</td>
<td>Similar to CS</td>
<td>Similar to MLBC</td>
</tr>
<tr>
<td>Nuclear detail</td>
<td>Clearly seen</td>
<td>Usually less clear as compared to MLBC</td>
</tr>
</tbody>
</table>

### Table 4: Comparison of 7 morphological parameters between the 100 Manual liquid based cytology and Conventional smear cases

MLBC Vs CS

In the present study the MLBC method was comparable to the CS since all the 66 cases reported benign in CS were also reported as benign on MLBC whereas all 34 cases reported as malignant on CS were also reported as malignant on MLBC (Fig 4,5). Thus the diagnostic accuracy of MLBC was similar to CS which was comparable with authors like Pawar PS et al. and Leung CS et al. MLBC and CS showed a sensitivity and specificity of 95.2% and 100% respectively whereas positive predictive value and negative predictive value of both methods were 100% and 96.9% respectively, which were comparable with other studies (Table 5).

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLBC</td>
<td>95.2%</td>
<td>100%</td>
<td>100%</td>
<td>96.9%</td>
</tr>
<tr>
<td>CS</td>
<td>95.2%</td>
<td>100%</td>
<td>100%</td>
<td>96.9%</td>
</tr>
</tbody>
</table>

### Table 5: Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of Manual liquid based cytology and Conventional smear

**Fig 4 CS (H&E, x200) - Duct Carcinoma**
ER was performed on 5 unstained MLBC smears as well on biopsy sections of the same 5 cases. On both MLBC smears and biopsy sections, ER was reported as negative (Fig. 7, 8, 9).

Unfortunately we were unable to get any cases where ER was positive on MLBC as well as on biopsy sections but several studies have demonstrated that LBC can be utilized for ancillary techniques such as ICC. Few studies have even shown that ER/PR ICC results correlate closely with the histological findings.27-30
VI. Conclusion

Fine needle aspiration is a safe and cost-effective method for the diagnosis of breast lesions. However, adequate preparation of cytological conventional smears determines the quality of FNA. Manual liquid-based cytology can be safely used in the diagnosis of breast aspirates. Liquid based cytology of breast aspirates provides better cellular preservation, less cell overlapping and elimination of blood and excessive inflammation compared to CS. Studies have shown a similar accuracy between MLBC and CS for the diagnosis of breast lesions. Thus MLBC on breast FNA can be an important adjunct to conventional FNA. In the present study we wish to stress the value of MLBC as a technique which can be as effective a diagnostic tool as CS in low-resource settings like India, which is a developing country and where the incidence of breast cancer is steadily on the rise. We observed in our study that the diagnostic accuracy of MLBC was similar to CS as was also reported by various other authors. Manual liquid based cytology can be used for ancillary tests such as ICC for hormonal receptors and molecular biology.

References


