

Comparative Study between Liquid Based Cytology and Conventional Smear in FNA Samples of Breast LesionsR. Kousalya¹, R. Padmavathi², Abu Arshad A³^{1,2,3} Assistant Professor, Department of Pathology, Government Mohan Kumaramangalam Medical College, Salem

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Conflict of interest: Nil

Abstract:

Introduction: Breast lesions are the most common lesions in women ranging from inflammatory lesions to malignant conditions. Fine-needle aspiration cytology is a safe and cost-effective, first line diagnostic tool in diagnosing breast lumps. FNAC helps in reducing the number of unnecessary surgeries in benign breast lesions.

Materials and Methods: This is prospective study includes 98 women patients and 2 male patients who are attending the FNA OP. Two samples were collected. One for CS preparation and other for LBC preparation. The following cytological parameters are compared. Cellularity, background material (blood, necrosis), informative background, monolayering, nuclear and cytoplasmic details.

Results: Cellularity in both the preparation showed moderate to high. In LBC preparation this is due to centrifugation of the collected sample. Clean background in LBC preparation. In CS method, there is bloody background which obscures the presence of the cells. Informative background is reduced or lost in LBC method. Whereas in CS method informative background is present. Monolayering is more in LBC method. Overlapping of cells is more common in CS preparation. Nuclear and cytoplasmic details are equally comparable in both the LBC and CS preparation.

Conclusion: Fine needle aspiration method is a safer and cost-effective method for the diagnosis of breast lesions. Liquid based cytology of breast aspirates provides a better cellular preservation, less cellular overlapping and elimination of obscuring background when compared to that of Conventional smears. With continuous experience Liquid based cytology may become the main FNA diagnostic method in future.

Keywords: Liquid based cytology, Conventional Smear, FNAC.

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Introduction

Breast lesions can be of various types from inflammatory to malignant. Some lesions are common in younger age group while others are more common in elderly age group. Benign lesions of the breast are the most common lesions which account for 90% of the clinical presentation related to breast [1]. Patients present with palpable breast lump and pain [2]. Breast lumps besides creating anxiety may result in carcinoma and deformity [3]. Breast tissue is under hormonal influence which causes changes in breast, present throughout life [4]. Fibro adenoma of the breast is the common cause of benign breast lump [5]. Breast cancer is the frequent cancer in women worldwide. Incidence rate differs worldwide from 27/100000 females in Eastern Africa to 96/100000 females in Europe. Breast cancer is common among women in India according to National cancer registry programme 2011 report. A woman has one in eight chance of developing breast cancer during her lifetime. By the year 2030 global burden of breast cancer will be more than two million every year. In India the

incidence of carcinoma of breast is increasing and the mortality rate for breast cancer in India is 11.1 per 10,000. Overall, breast nodules are more common in women.

Fine-needle aspiration cytology is a safe and cost-effective, first line diagnostic tool in diagnosing breast lumps. FNAC helps in reducing the number of unnecessary surgeries in benign breast lesions. For many years, conventional smears have been regarded as the gold standard technique for diagnosing breast lesions in cytology. From the aspirated material, smears of variable number are prepared. It consumes time and has been tedious for the cytologists to screen the slides.

Technical aspects add to the problem, which include improper smear preparation and fixation leading to poor preservation of cellular details. Thick smears, cellular overlapping and obscuring inflammatory infiltrate all interfere in reporting the smears.

To overcome the above disadvantages posed by conventional method, the Liquid-based technique has been used with increasing frequency worldwide in most of the centers for gynecological as well as non-gynecological samples. Two systems named Thin-prep and Sure-path approved by US Food and Drug Administration (FDA) are commonly used. Both represent first generation liquid based cytology systems, and they consist of automated equipment's, filters, plastic containers, and vacuum devices. When compared with conventional smear, the cost of preparing slides using the above two systems was increased to a greater extent, making the improved method potentially inaccessible. Liquid-Prep TM, the second generation technique is a simpler one. It requires low cost, because most of the automated machines and devices are not used in this liquid based cytology system. It also enhances clear visualization due to monolayer spread of cells.

We planned to use a commonly available instrument (centrifuge), and to prepare slides from fine needle aspirates. The results are interpreted using standard morphologic criteria proposed for liquid based smears. Finally, the left over liquid based sample can be used for ancillary tests such as immunocytochemistry and molecular studies. Based on this aim of our study is to compare liquid based cytology technique with conventional smear method and to correlate with histopathological diagnosis Also to compare the cyto-morphological features of manual liquid based preparation with conventional cytology in breast lesions using FNA technique.

Materials and Method

This study was done in a tertiary care teaching hospital, in a total of 100 breast fine needle aspirates obtained prospectively from patients who

come to our pathology department with breast lump during the study period of one year. Both female and male patients with age ranging from 18 to 80 years and patients with clinically palpable breast enlargement were included in our study, while uncooperative patients. While uncooperative patients with age less than 18 years were excluded. Both male and female patients with palpable breast lesions are included in the study. Patient's age, clinical history and ultrasound findings (if available) were recorded. Consent from the patient was obtained. Conventional and liquid based smears were prepared using cytological material obtained by separate needle passes. The aspirates were performed using 26 gauge needle connected to 10ml syringe. Non-aspiration technique was followed to minimize bloody samples. Liquid based cytology smears were prepared using centrifuge machine. Conventional smears were stained in Hematoxylin and Eosin and the original cytological diagnoses were made using conventional slides.

Stained conventional and liquid based smears were interpreted using the diagnostic categories recommended by E.C. Working Group on Breast Screening. The sensitivity, the specificity, the diagnostic accuracy, the positive predictive value and the negative predictive values were analyzed for liquid based smears and they were compared with the conventional one. In addition, both liquid based and conventional methods were correlated with histopathological diagnosis whenever available. Corresponding mastectomy specimens were received and fixed in 10% formalin. Paraffin embedded sections obtained from routine processing were cut at a thickness of 3microns using Leica microtome. The slides were then stained with Hematoxylin and Eosin. Cytological morphological features were given scores based on the findings as below.

Table 1: Cytomorphological scoring

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 recognised	Partially recognised	Well recognised	
Nuclear detail	Poor	Fair	Good	Very good
Cytoplasmic detail	Poor	Fair	Good	Very good

Results

Total of 100 fine needle aspiration samples (98 from women and 2 from men) were included in the study. Both conventional and liquid based smears were prepared for all 100 fine needle aspiration specimens. We first categorized based on cytological diagnosis and in our study benign category includes fibro adenoma, fibrocystic

disease of breast and gynecomastia constituting about 35% of cases in each method. Suspicious of malignant category constitutes 2% cases in LBC preparation and 1% case in conventional method.

Malignant category had equal incidence in liquid based and conventional methods, constituting 61 cases of ductal carcinoma and 2 cases of mucinous carcinoma. In benign lesions most

common age group was 20-30 years and in malignant lesions it was 51-60 years. Coming to cytomorphological correlation we started with Cellularity and in liquid-based method adequate was 33 in number and abundant was 66 in number, while in conventional method, adequate was high with 93 in number. Next we did comparison of background material (blood, cell debris) between Liquid based and Conventional method and in LBC there was zero findings in all samples while in CM there was occasional (n=16), good amount (n=40) and abundant (n=44) in the samples. Comparison of Informative background between Liquid based and Conventional method showed

that present in 66 in conventional method and 30 in LBS method. Comparison of monolayer arrangement between Liquid based and Conventional method had findings of 14 in occasional and good amount was 86 in LBC method and it was 14 and 16 respectively in CM while there was no finding in 75 samples. Next with cell architecture it was partially recognized in 14 and well recognized in 86 in LBC while it was 26 and 74 in conventional method. Comparison of nuclear detail showed 14 as fair and 86 as good in LBC while it was 18 and 82 in conventional method. In cytoplasmic details in both method all samples (n=100) showed good level of findings.

Table 2: Diagnostic ability of Liquid based cytology method on different HPE findings

	Benign	Malignant	FA	FCD	GM	SM	DC	MC
Sensitivity	100%	100%	100%	100%	100%	100%	100%	100%
Specificity	95.45%	100%	97.18%	98.96%	100%	98.99%	100%	100%
Positive Predictive Value	91.42%	100%	93.55%	75%	100%	50%	100%	100%
Negative Predictive Value	100%	100%	100%	100%	100%	100%	100%	100%
Accuracy	96.93%	100%	98%	99%	100%	99%	100%	100%

On further evaluation Among 100 fine needle aspiration cases, it was possible to compare cytology results with mastectomy specimens in 36 cases only. Correlating LBP with available histological diagnoses, the following results were inferred. Out of 10 cases of fibroadenoma, all the 10 correctly diagnosed. 1 case of FCD correctly interpreted. 1 case of GM correctly diagnosed. 1 case of SM in LBC diagnosed as fibroadenoma in histopathology and another case of SM in LBC preparation diagnosed as ductal carcinoma in

histopathology. Totally 33 cases of Conventional preparation were correlated with histopathology findings. Malignant category of conventional smears correlated very well with histopathological diagnosis. Out of 10 cases of fibroadenoma 8 cases correlated with histopathology. 2 cases of fibroadenoma were not diagnosed in CS method due to very low cellularity. Gynecomastia and fibrocystic change cases of conventional smear preparation correlated very well with histopathology findings.

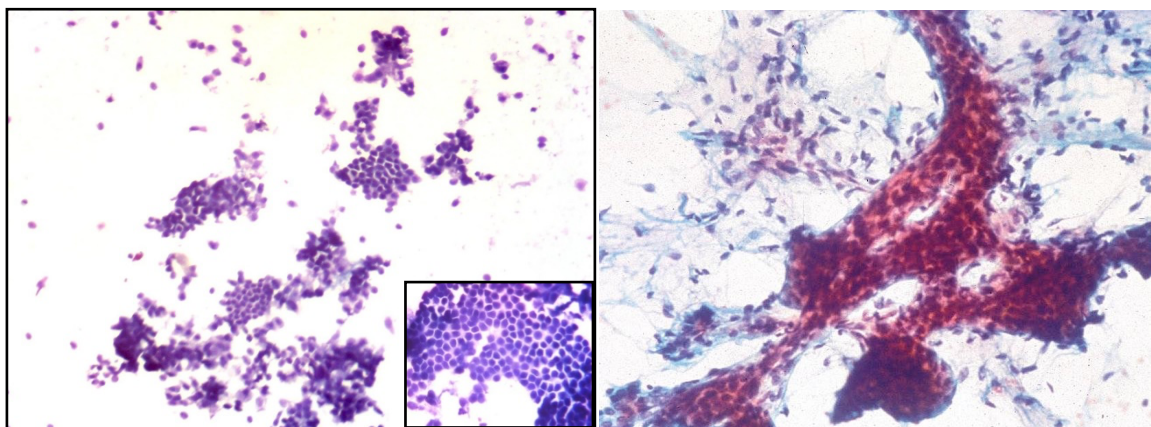


Figure 1: Fibroadenoma – Liquid based smear vs Conventional smear

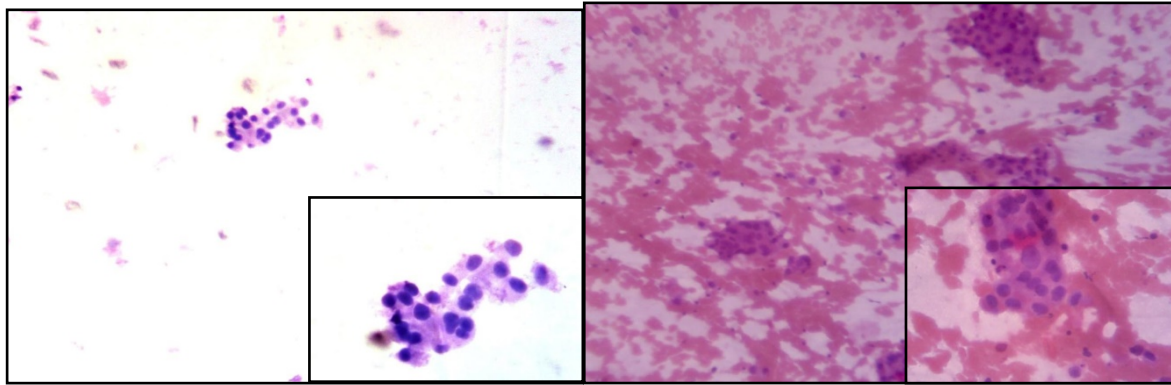


Figure 2: Ductal carcinoma Liquid based smear Vs Conventional Smear

Discussion

Over the past two decades, liquid based method has emerged as a newer technique in the field of cytology. Even though this technique has been in routine use at many diagnostic centers, it has not completely replaced the conventional method. The opinion regarding the best method is still controversial among the cytopathologists. The advantages offered by liquid based preparations include less number of slides to be screened, uniform cellular layer, clean bloodless background, better preservation of cell morphology.

In view of the above suggestions, in this study, liquid based sample was collected from a separate needle pass. The lytic agents added to collecting media allowed the sample to be of better quality with less obscuring background elements than that of the conventional smears which are thick with obscuring blood and inflammatory cells. The nature of the liquid based processing technique allows a thin layer of representative sample to be deposited on liquid based slide in a well-defined area and enables cytologist to screen the slides at a faster rate.

Coming to cellularity, in a broader terminology, sample is said to be adequate if it is cellular and of good quality with well-preserved cellular morphology. Also it should be, representative of the lesion. Dey P et al [6], reported the cellularity in LBC was equal to conventional preparation. Gerhard et al [7], Ryu et al [8] described that cellularity in LBC and conventional preparations was same for both. Michael et al [9] and Leung et al [10] reported that the cellularity in LBC preparations is slightly inferior or superior to the conventional smears. Ryu et al [8] and Jose et al [11] both described that the cellularity between the LBC preparation and the conventional smear preparation are same. Almost all the above studies mentioned that the cellularity in both the LBC and conventional smear preparation are equal.

In the present study almost all the cases in LBC showed moderate to high cellularity except very few cases which showed low cellularity. For those

cases which showed low cellularity second slides were made with the remaining material and the diagnosis given. This is one of the advantages in LBC preparation. Conventional smears showed moderate cellularity in most of the cases. Very few of the cases showed low cellularity. In low cellularity cases when opinion was not easily made another prick was done to give the opinion.

In two cases where the conventional smear showed scant cellularity LBC slides show adequate cellularity (possibly due to centrifugation). In our study, the cellularity in both LBC and CS preparations was almost equal. This is in concordance with the above studies.

In our study, most of cases of LBC preparation showed clean background with absence of blood, cell debris and necrosis in the background. This is one of the advantages of the LBC preparation which helps in easy screening. In CS preparation most of the cases show bloody material in the background which obscures the cells. This is one of the disadvantages of the CS method. Veneti et al [12], Dey P et al [6] and many authors described that the informative background was lost in LBC preparation which is one of the disadvantages in diagnosing the benign cases like fibroadenoma and malignant cases like mucinous carcinoma. Gerhard et al [7], Dey P et al [6] describe that the background material like blood and necrosis are lost in LBC preparation, which gives clean background and helps in easier screening. This is in concordance with the above studies.

Informative background is one of the most important clues in diagnosing the lesions in cytology preparation. In our study informative background was found to be reduced but not lost in cases of LBC. This is one of the disadvantages in LBC method as described by many authors [13] but in CS method informative background is preserved which helps in diagnosis.

In the present study cell architecture was well recognized with LBC(86/100). Conventional smear showed well recognizable architecture in 74/100 cases. This is probably due to there being less

overlapping of cells in LBC, which resulted in better assessment of cell morphology and architecture in LBC method. Many authors described the same features [14]. Nuclear and cytoplasmic details are of equally good quality in almost all cases in both LBC and CS method the benign category included in the present study is fibro adenoma and fibrocystic disease of breast and gynecomastia. They constitute about 30 %, 4% and 2% respectively of all the breast lesions diagnosed in conventional and LBC methods. Current study showed that the diagnostic accuracy for LBC and CS preparation are 97% and 94 % respectively. These values imply that our study results are almost equal to that observed in many studies. The sensitivity and specificity in our study is 100% and 96%.

LBC preparation in fibroadenoma showed benign looking ductal epithelial cells, arranged in sheets, small clusters and three dimensional clusters. Some of the cases showed staghorn clusters. Isolated myoepithelial cells are seen. Most of the cases showed loss or paucity of the stromal elements like fibromyxoid stroma. Benign looking ductal epithelial cells without increase in nuclear cytoplasmic ratio arranged in small clusters and isolated myoepithelial cells helps us to diagnose fibroadenoma, even though there is loss or paucity of fibromyxoid stroma in the background. Continuous practice helps one to diagnose fibroadenoma.

Mygdakos et al [15], Michael et al [9], Leung et al [10] and many other authors also observed decrease in myoepithelial cells and paucity or loss of stromal elements in fibroadenoma cases. Ryu et al [8] has interpreted some of the breast lesions, which showed false increase in ductal epithelial cells due to decrease in the fibromyxoidstroma and myoepithelial cells, thus misdiagnosing these cases as suspicious for malignancy. Leung et al [10], Perez et al [16], Kollur et al [17] and many authors encountered same problem. We also encountered a similar problem, but upon review of the doubtful cases, we could identify the predominance of cell clusters arranged in small clusters and three dimensional clusters without crowding or overlapping. Even though there is loss or paucity of background material, presence of uniform cell morphology, without increase in nuclear cytoplasmic ratio and the arrangement helps us to diagnose fibroadenoma in LBC.

In conventional preparation the diagnosis of fibroadenoma is easily done because of the staghorn arrangement of the cells with myoepithelial cells and background fibromyxoid stroma. But some cases are difficult due to the bloody background, the nuclear features are not seen clearly and the whole slide has to be searched. Two to three slides might have been made and all

have to be screened, which consumed more time when compared to LBC were most of the cases are reported with a single slide. Two cases in conventional preparation had insufficient material to interpret.

The diagnosis of fibrocystic disease of breast by CS and LBP preparation shows similar features in both methods. Ductal epithelial cells and scattered apocrine metaplastic cells. But the cellularity in the conventional preparation is low. So another prick is usually done to diagnose the case. But LBC preparation shows moderate cellularity in such cases due to centrifugation which helps in diagnosing the case, one of the advantages of LBC.

Gynecomastia cases in both the method showed ductal epithelial cells. In CS preparation cellularity is low to moderate, so two to three slides are needed to report whereas in LBC, the cells are subjected to centrifugation and the diagnosis is made with a single slide itself.

Two cases of the category, suspicious of malignancy were encountered in our study. One case in LBC method showed small clusters, three dimensional clusters and singly scattered epithelial cells with moderate amount of cytoplasm and nucleus showing mild pleomorphism. Features in CS preparation showed sheets of epithelial cells and with nucleus showing mild pleomorphism. On histopathological correlation one of the two turned out to be fibroadenoma, other case was diagnosed as ductal carcinoma. Bedard et al described that in their study 75% and 71% of the category suspicious for malignancy in CS and LBC preparation turned out to be malignant in histopathology study.

Most of the breast carcinomas are easily diagnosed with the help of FNA. Veneti et al [12], Biscotti et al [18], Ryu et al [8] described that both the types of cytological preparations CS and LBC preparations have comparable features for detection of ductal carcinomas. Dey et al [6] stated that it was easier to diagnose the ductal carcinoma in LBC preparation because of the clean background. They also described that the clean background means uninformative background because main features of carcinomas like blood and necrosis are lost in LBC preparations. The results of the present study are in concordance with the observation made by most of the above authors. The sensitivity, specificity and the diagnostic accuracy for ductal carcinoma in CS and LBC preparations showed 100%.

LBC preparation showed malignant ductal epithelial cells arranged in three dimensional clusters, small clusters and also singly scattered in a clean background. Cells have scant to moderate amount of cytoplasm with nucleus showing marked pleomorphism. Most of the cases show fine

chromatin. CS preparation showed sheets of ductal epithelial cells in the background of blood. Nucleus features are almost the same for both preparations as described by Dey et al [6], Ryu et al [8] and many authors. But cytology preparation may not help to categorize the ductal carcinomas which are a major disadvantage of all FNA samples of both LBC and CS preparations.

Two cases of mucinous carcinomas were encountered in our study. Komatsu et al detected mucinous carcinoma in a single case by the presence of mucous. Michael et al [9], Veneti et al [12] and many other authors described that the mucinous carcinomas diagnosed by cytology depends largely on the presence of mucous in the background, which can be reduced or lost in LBC preparation. But this feature is preserved in CS preparation. In our study both the cases in LBC preparation revealed 3D clusters of malignant ductal epithelial cells with the background showing focal areas of mucous and entrapped capillaries. This helped to make diagnosis as mucinous carcinomas. One of the cases was confirmed with histopathological diagnosis. In CS preparations background mucus is preserved with sheets of malignant ductal epithelial cells which makes it easier to interpret. Our study is in concordance with the study by the above authors.

Conclusion

Fine needle aspiration method is a safer and cost effective method for the diagnosis of breast lesions. However, proper preparation of cytological smears determines the quality of diagnosis. Liquid based cytology of breast aspirates provides a better cellular preservation, less cellular overlapping and elimination of obscuring background when compared to that of Conventional smears. But the loss or paucity of informative background is a disadvantage in Liquid based cytology. Studies have shown similar accuracy between Liquid based cytology and Conventional smear for the diagnosis of breast lesions. Thus, Liquid based cytology can be used as important ancillary technique along with the Conventional smear for diagnostic accuracy.

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