

Study of ductal lavage cytology in women at high risk for breast carcinoma



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ABSTRACT

Background: Ductal lavage (DL) involves cannulating the nipple aspirate fluid (NAF)-producing ducts with a microcatheter and lavaging with saline to retrieve cells for cytological and molecular analysis. This method improves upon NAF and fine-needle aspiration and can provide important data on cellular yield and molecular markers in DL fluid. **Aims and Objectives:** The present study aims to evaluate a non-invasive method called DL for obtaining epithelial cells from breast ducts of women at risk for breast cancer, to study the cytology of DL in women at risk of/clinicoradiological possibility of breast carcinoma, and to correlate the cytology of DL with Gail risk score and with histopathology (wherever available). **Materials and Methods:** The present study, conducted at Hindu Rao Hospital, Delhi, between 2012 and 2014, examined 36 patients with nipple discharge. Nipple aspiration and DL were performed, and the results were analyzed and compared. The present study also looked at the correlation between DL results and the patient's Gail score and correlated histopathology with DL cytology. **Results:** The present study enrolled 36 women and studied 50 ducts from 46 breasts. Nipple fluid was aspirated from 34 patients, with inadequate cellular material for diagnosis in 24 breasts, benign in 18, atypia in 2, and no malignancy found. The correlation of DL with Gail score was statistically significant, as was the correlation between epithelial cells in DL and NAF samples. The correlation between DL and histopathological findings was statistically insignificant. **Conclusion:** DL samples may miss atypical cells, and have higher epithelial cell count than NAF. Higher Gail score correlates with mild atypical cells in DL ($P=0.003$).

Key words: Duct lavage; Breast cancer; Cytology; Nipple aspirate fluid

INTRODUCTION

Nipple discharge is common in women and accounts for 5% of breast-related symptoms.¹ About 50–80% of women experience some form of nipple discharge, with 6.8% of women presenting to a clinician for breast disorder.² The breast consists of ductal systems lined by epithelial and myoepithelial cells, with most breast cancers arising from the epithelial lining.^{3,4} The normal secretions of the breast are milk and colostrum produced during pregnancy and postpartum. Concern arises when nipple discharge is spontaneous and unilateral, with milky discharge called galactorrhea and commonly caused by pituitary adenoma, hypothyroidism, and

other factors. Bloody or serous discharges are commonly due to benign lesions but may rarely be due to malignancy. The most common causes of nipple discharge are duct papilloma, cysts, or carcinoma, with cancer rarely being the cause in the absence of a palpable mass or mammographic abnormality.^{5,6} The main objective in evaluating patients with nipple discharge is to identify the underlying cause and differentiate between benign and high-risk lesions. Nipple aspirate fluid (NAF) may be used for analysis but has limitations.

Aims and objectives

To study the cytology of DL in women at risk of/ clinicoradiological possibility of breast carcinoma and to

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correlate the cytology of DL with Gail risk score and with histopathology (wherever available)

MATERIALS AND METHODS

This prospective interventional study was conducted at the Department of Pathology in collaboration with the Department of Surgery, Hindu Rao Hospital, Delhi-110007 from May 01, 2012, to April 30, 2014. Thirty-six patients with nipple discharge with or without lump were included in the study, and a total of 50 ducts were studied from 46 breasts. Written informed consent was obtained from all subjects, and the protocol was approved by the institutional review boards/ethics committees.

Inclusion criteria

The presence of nipple discharge with or without breast lump and Gail score $>1.7\%$.

Exclusion criteria

Women with surgery within 2 cm of nipple, women taking tamoxifen, women allergic to lignocaine jelly, age <18 years, and unwilling patients.

The patient was prepared for ductal lavage (DL) using a Neoflon 24G catheter and lidocaine jelly (Figure 1). The collected samples were analyzed using diagnostic categories similar to the 1997 criteria for breast fine-needle aspiration biopsy samples. Cell numbers were quantified by counting epithelial cell clusters and single cells. DL and nipple aspiration results were compared and correlated with the Gail score. Histopathological samples from 12 breasts were processed and stained for analysis, with fibroadenoma, benign breast lobule, and fibrocystic diseases considered benign for correlation purposes.

RESULTS

Demography and clinical features

The study enrolled 36 women and examined 50 ducts from 46 breasts, aged 25–56 years (mean age=38.33 years, SD=7.91). Most patients (35–40 years) were in their mid-30s. Four patients had a positive family history of breast carcinoma (11.11%). The majority of patients (94.44%) had menarche before age 15. Ultrasonography detected benign lesions in all 28 patients who underwent the procedure. Among 12 patients who underwent both ultrasonography and biopsy, all had benign lesions. Six women had a 5-year Gail risk of breast cancer of 1.7% or higher, calculated using the Gail model, which is available on the National Cancer Institute website. Thirty patients reported nipple discharge as their chief complaint, while six had both nipple discharge and a breast lump.



Figure 1: Patient undergoing procedure of ductal lavage in OT

Table 1: Cytological findings in ductal lavage

Ductal lavage	Number of cases	Percentage
Cells		
Macrophages	34	68.00
Giant cells	6	12.00
Squamous Cells	8	16.00
Background		
Proteinaceous	44	88.00
Hemorrhagic	6	12.00
Number of epithelial cells		
<10	14	28.00
≥ 10	36	72.00
Cluster	28	56.00

Table 2: Diagnosis in ductal lavage samples

Ductal lavage diagnosis	Number of ducts	Percentage
Inadequate	14	28.00
Benign	32	64.00
Mild atypia	4	8.00
Malignant	0	0.00
Total	50	100

Nipple discharge

Nipple discharge was present in 36 patients: 14 (38.89%) from the left breast, 12 (33.33%) from the right breast, and 10 (27.28%) from both breasts. Discharge was spontaneous in 24 patients and non-spontaneous in 12 patients, only appearing upon pressure. Cannulated ducts showed four different discharge colors: greenish (48% of ducts), milky (32%), dirty white (16%), and bloody (4%). The color of discharge was not reported for uncannulated ducts. We correlated the color of discharge with cytological findings only in cannulated ducts.

DL

Surgeons achieved successful cannulation in 50 of 62 ducts (80.64%). Unsuccessful attempts were due to the inability to cannulate or fully seat the catheter. Mean volumes of normal saline infused and effluent collected were 14 mL and 5 mL, respectively. Cytological samples

Table 3: Ductal lavage and Gail score correlation

Ductal lavage	Number of cases	Gail score (%)		P-value (Overall)	P-values		
		Mean	±SD		Inadequate versus benign	Inadequate versus mild atypia	Benign versus mild atypia
Inadequate	8	0.73	0.25	0.034	1.000	0.044	0.048
Benign	26	0.92	0.70				
Atypia	2	2.60	0.00				

Table 4: Cytological findings in nipple aspirate fluid

Nipple aspirate fluid cytology	Number of breasts	Percentage
Cells		
Macrophages giant cells	30	68.18
squamous cells	0	0.00
	4	09.09
Background		
Proteinaceous	40	90.91
Hemorrhagic	4	09.09
Number of epithelial cells		
<10	24	54.55
≥10	20	45.45
Clusters	12	27.27

Table 5: Diagnosis of nipple aspirate fluid samples

Diagnosis (nipple aspirate fluid)	Number of breasts	Percentage
Inadequate	24	54.55
Benign	18	40.91
Mild atypia	2	4.55
Malignant	0	0.00
Total	44	100

were obtained from 50 ducts in 36 subjects. Two patients had 4 discharging ducts, 2 patients had 3, 12 had 2, and 18 had 1. Single duct was cannulated in 22 patients, and two ducts were cannulated in 14. The majority (72%) of samples had adequate cellular material for diagnosis, with a median of 25 epithelial cells, scattered singly, or in clusters of at least 10. Inflammatory cells, including neutrophils, lymphocytes, and foamy macrophages, were also present, with foamy macrophages being the most common (68%). Giant cells were seen in 12% of ducts and squamous cells in 16%. Smears showed proteinaceous background in 88% and hemorrhagic background in 12%. Mild atypia was seen in 8% of ducts (Figure 2). Final diagnoses were inadequate cellular material (14 ducts), benign cells (32 ducts), and mild atypia (4 ducts); no malignancy was found. The mean age of menarche was 13 years in benign cases and 12 years in cases diagnosed as inadequate or atypical. The correlation of DL diagnosis with Gail score showed a statistically significant P=0.034 overall. Inadequate versus benign samples were statistically insignificant, but inadequate versus mild atypia and benign versus mild atypia were

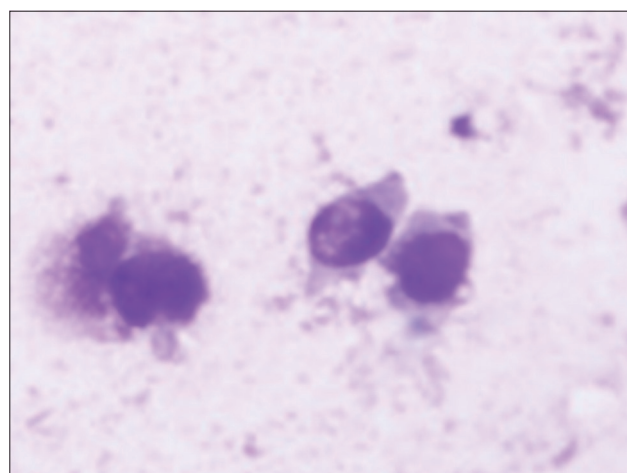


Figure 2: Ductal lavage showing ductal epithelial cells along with cystic macrophage (arrow). (MGG stain, ×40). Inset showing ductal epithelial cells with mild atypia having mild anisonucleosis, mild nuclear enlargement and prominent nucleoli. (MGG stain, ×100)

statistically significant. Higher grade of DL cytology was considered for correlation in patients with more than one duct cannulated (Tables 1-3).

NAF

Nipple fluid was collected from 34 patients, excluding 2 postmenopausal women who could not provide enough fluid. The NAF cytology results are presented in Table 4 and Figure 3. Macrophages were the dominant cell type and were present in 68.18% of samples. Only 27.27% of samples contained clusters of 10 or more epithelial cells, while most had smaller numbers of epithelial cells. The mean epithelial cell count in NAF was 35.64. Inadequate cellular material for diagnosis was found in 24 breasts, benign cells in 18 breasts, and atypia in 2 breasts. No malignancy was detected (Table 5). More than half (54.55%) of subjects had NAF samples with inadequate cellular material or unacceptable technical quality.

DL and NAF

DL was examined in 50 ducts from 46 breasts and NAF in 44 breasts. To correlate their cytology, we considered the higher grade of DL cytology in breasts with multiple cannulated ducts. Cytology correlation revealed a significant P<0.001 (kappa=0.225) (Table 6). Epithelial cells median

Table 6: Ductal lavage and nipple aspirate fluid correlation

Ductal Lavage→NAF↓	Inadequate		Benign		Mild atypia		Total
	Number of breasts	%	Number of breasts	%	Number of breasts	%	
Inadequate	8	66.67	14	50.00	2	50.00	24
Benign	4	33.33	14	50.00	0	0.00	18
Atypia	0	0.00	0	0.00	2	50.00	2
Total	12	100	28	100	4	100	44

P<0.001, Kappa<0.225

Table 7: Correlation of ductal lavage and histopathological findings

Ductal Lavage→Histopathology↓	Inadequate		Benign		Mild atypia		Total
	Number of breasts	%	Number of breasts	%	Number of breasts	%	
Benign	2	100.00	6	75.00	0	0.00	8
Usual hyperplasia	0	0.00	2	25.00	2	100.00	4
Total	2	100	8	100	2	100	12

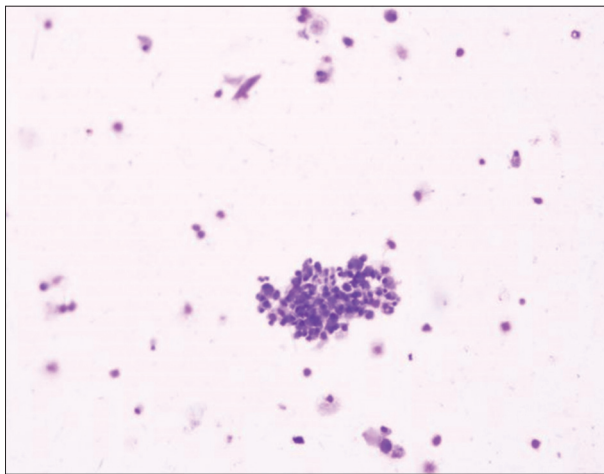


Figure 3: NAF showing ductal epithelial cells in small cluster against a proteinaceous background (MGG stain, 10 xs)

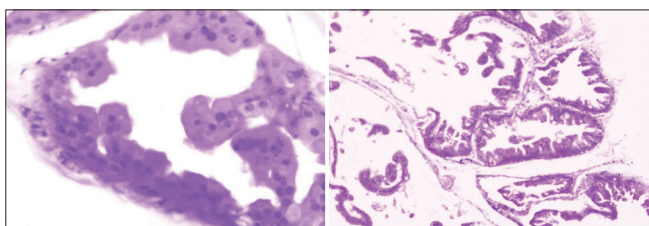


Figure 4: Histopathological examination of breast showing usual ductal hyperplasia (H&E, 40 xs). Inset showing usual ductal hyperplasia (H&E stain, 100 xs)

was 25 (range 0–350) for DL and 8 (range 0–350) for NAF. The Mann–Whitney test yielded a significant P=0.035 (Mann–Whitney test value=824.00) for epithelial cells correlation.

Histopathological examination

Histopathological examination was available in 12 breasts only, 8 of which show benign histological features and

4 breasts show findings of usual ductal hyperplasia (Figure 4).

The correlation of DL findings and histopathological findings was done using the Chi-square test. P=0.072 which was statistically insignificant (Table 7).

DISCUSSION

Patients’ age range in the study was 25–56 years with a mean of 38.33 years. The mean age was 52 years in Dooley et al.⁷ and Francescatti et al.⁸ study and 50 years in Khan et al.⁹ study. In Fabian et al.¹⁰ study, the mean age was 44 years with most patients between 30 and 60 years of age, and 60% of women were premenopausal. Four (11.11%) patients had positive family history of breast carcinoma, all of them had benign epithelial cells. The mean menarche age for patients showing mild atypical and benign epithelial cells was 12 and 13 years, respectively. The successful duct cannulation rate was 80.64% in the present study and 87% in Khan et al.⁹ study. In the present study, 72.22% of patients had one-sided breast involved with the left breast involved more than the right breast. DL cytology showed epithelial cell clusters in 56% of cannulated ducts, and the majority of DL samples showed foamy macrophages. The percentage of women who were NAF yielders in the present study was 94.44% and was higher than the percentage of women yielding NAF in Dooley et al.⁷ study. Two women were found to be non-yielders of NAF in the present study.

Limitations of the study

Cost of Ductal lavage procedure related materials is substantially higher than NAF and RPFNA. As it is an OT procedure, so it requires long hospital stay for the patient.

CONCLUSION

The study included 36 cases of nipple discharge, with the cytological evaluation of DL and NAF samples. Benign cells were found in 64% of ducts, while malignancy was not detected in any ducts. A statistically significant correlation was found between DL diagnosis and Gail score. In NAF samples, inadequate cellular material for diagnosis was found in 54.55% of breasts, and malignancy was not detected in any cases. There was a statistically significant correlation between the cytology of DL and NAF. Histopathological examination was available in 12 breasts, with benign features in 8 and usual ductal hyperplasia in 4, but there was no significant correlation between DL findings and histopathological findings.

The study indicates that DL samples may not always detect atypical cells and that the epithelial cell count is higher in these samples than in NAF samples. In addition, there is a correlation between an increased Gail score and the presence of mild atypical cells in DL samples ($P=0.003$).

Recommendations

- To determine the sensitivity and specificity of DL as a breast cancer screening tool, further studies involving a larger number of cases should be conducted
- Future studies on DL should include more patients over a longer period of time to gather more data on its utility as a screening method for breast cancer
- The use of newer techniques such as cytospin and Millipore filtration should be explored to increase the cell count in DL samples.

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MC- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article; **SS-** Design of study, concept, clinical protocol, manuscript preparation; **DSC-** Literature survey and preparation of figures, coordination, manuscript editing and revision.

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