

# Nipple Discharge and Early Diagnosis of Breast Cancer

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## ABSTRACT

Nipple discharge is one of the most common breast complaints in women. Study was designed to see the variation in biochemical parameters like carcino-embryonic antigen (CEA), Lactate dehydrogenase (LDH), cholesterol, triglyceride and occult blood in malignant and non-malignant discharge. Besides, the effect of allopathic/homeopathic drug on malignant nipple discharge was also studied. Color of normal fluid was milky white and of malignant was dark brown. Levels of CEA and LDH were markedly increased in malignant discharge than in non-malignant. In contrast, the level of protein, cholesterol and triglyceride were decreased in malignant discharge as compared to non-malignant. Incubation of drugs with malignant fluid shows no marked electrophoretic changes in protein pattern. On the other hand, incubated mixture run on reverse phase HPLC showed that tamoxifen is most effective than methotrixate. Results showed that analysis of nipple discharge may help in the diagnosis and treatment of breast cancer. Hence, further research is needed on large number of samples to reach a definite conclusion.

**Key Words:** Nipple discharge; HPLC; Methotrixate; Tamoxifen

## INTRODUCTION

Nipple discharge is one of the most common breast complaints in women. Galactoria (milky discharge) may occur during pregnancy or breast-feeding or as a result of drug therapy etc. Non-bloody discharge is most common and is usually benign. Bloody discharge is usually considered a sign of cancer until proven otherwise (State, 1991). The cause of discharge found to be intraductal papillomas and cystadenopapillomas (Martynink *et al.*, 1999). Clinical examination showed the discharge to be arising from one or several of Montgomery's areola tubercles, with the breast lump localized to the subareolar region immediately beneath the discharging tubercles (Walkins *et al.*, 1989).

This paper reports observations on biochemical parameters (carcino-embryonic antigen, CEA; Lactate dehydrogenase, LDH; cholesterol, triglyceride and occult blood) in malignant and non-malignant discharge; changes in nucleus, if any; and effect of allopathic/homeopathic drug on malignant nipple discharge.

## MATERIALS AND METHODS

Nipple discharge of normal and breast cancer patient was collected manually. Cell cytology of non-malignant and malignant discharge was performed. CEA, LDH, protein, cholesterol, triglyceride were performed by standard kits (Merck and Sigma). Electrophoresis of discharge fluid was performed by the method of Laemmle (1970) using 12.5% gel electrophoresis. Effect of drugs like methotrixate (12 mg), tamoxifen (20 mg) and a homeopathic drug was studied using reverse phase HPLC. It was performed on C18 column of Vydac (3.9 x 300 mm). These drugs were

incubated at 50°C for three days with nipple discharge (*in vitro* study) and observed their effect on protein of different molecular weights by gel electrophoresis. DNA of nipple discharge was identified using the PCR technique using 0.8% agarose gel.

## RESULTS AND DISCUSSION

Color of normal fluid was milky white and of malignant was dark brown. Cell cytology of nipple discharge shows number of lymphocytes and no malignant cell. On the other hand, cell cytology of milky discharge (considered as normal) showed few lymphocytes. Bauer *et al.* (1998) observed the physical features of malignant discharge like brown color and malignant cells. The results of present indicated that occult blood was found only in malignant discharge, thus supporting the studies of Ito *et al.* (1997) who has also observed occult blood in secreted fluid. Level of CEA in malignant fluid was more than normal. Increased level of CEA was also reported by Okura (1996) and Ito *et al.* (1997). They found that elevated CEA in nipple discharge is indicative of non-palpable breast cancer. Whereas, Okazaki (1999) observed that increased level indicate latent malignancy. Level of LDH was also

**Table I. Variation of biochemical parameters in non-malignant and malignant discharge**

Parameters	Non-malignant	Malignant
CEA ( $\mu\text{g mL}^{-1}$ )	5.0	10.0
LDH ( $\mu\text{L}^{-1}$ )	50.0	120.0
Protein ( $\text{mg dL}^{-1}$ )	20.0	10.0
Cholesterol ( $\text{mg dL}^{-1}$ )	80.0	40.0
Triglyceride ( $\text{mg dL}^{-1}$ )	45.0	20.0
Occult blood (%)	25.0	Nil

increased in malignant discharge as compared to normal. Similar observation have been reported by Kawamoto (1994) that the increased LDH level in nipple discharge may be an indicative of non-invasive cancer.

Level of protein was found to be 10 mg mL<sup>-1</sup> in malignant and 20 mg mL<sup>-1</sup> in non-malignant discharge. No data in literature is available on the level of protein in discharge. Levels of triglyceride and cholesterol were 45 and 80 mg dL<sup>-1</sup> in non-malignant discharge. In malignant discharge, the level of triglyceride and cholesterol were 40 and 20 mg dL<sup>-1</sup>. Ogan *et al.* (1944) also observed increased levels of these lipids in the non-malignant nipple discharge. State (1991) found increased level of lipids in non-malignant discharge, which may be due to pregnancy, lactation, drug therapy or due to thyroidism. There may be a change in physiological process of breast that may increase the level of fat.

<sup>1</sup>Electrophoresis of both non-malignant discharge and malignant discharge showed no difference in electrophoretic protein bands. Protein of approximately 90-14 kda was observed in both discharges.

All drugs were incubated with malignant discharge for one, two and three days. Incubated discharge was analyzed by 12.5% SDS polyacrylamide gel. Best results were observed on two days incubation. Malignant discharge with homeopathic drug showed that the density of 67 kda was markedly decreased. In case of methotrixate a protein of 55 kda may be absent or in less amount. On the other hand, in case of tamoxifen no difference was seen.

Incubated mixture was also analyzed by reverse phase <sup>1</sup>HPLC. It was observed that in non-malignant fluid there is a small peak (rt=11.81) and long peak (rt=14.58) in malignant. The malignant fluid profile is different than non-malignant fluid. Here a broad peak (not well separated) split into 2 with rt 8.80 and 10.43. Besides, a sharp peak at 14.19 rt was also observed. Malignant fluid incubated with tamoxifen shows one sharp peak and one small peak with retention time (rt) 14.13 and 15.13. With methotrixate, one broad peak split into 2 has a rt of 9.38 and 10.52. Besides a small sharp peak with rt of 14.28 was also observed. Along with homeopathic drug three peaks, one small and two long with rt 16.74, 18.38 and 20.71 were also observed. Electrophoresis of DNA of both malignant and non-malignant discharges on 0.8% agarose indicate a band of approximately 100 kb. Results show that tamoxifen was more effective than methotrixate. It may be explained that tamoxifen is non-steroidal estrogen and it is more effective

in estrogen receptor positive patients. Results of homeopathic drug cannot be predicted accurately.

## CONCLUSION

It was concluded that analysis of nipple discharge may help in the diagnosis and treatment of breast cancer. It is, therefore, suggested that inspection and palpation for nipple discharge should be a part of every breast examination. A critical analysis should be made of every nipple discharge with the ultimate objective being differentiation between benign and malignant discharges. Hence, further research is needed on large number of samples to reach a definite conclusion.

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<sup>1</sup> Figures were not sharp enough for printing