Diagnostic Values of Carcinoembryonic Antigen, Cancer Antigen 15-3 and Cancer Antigen 125 Levels in Nipple Discharge

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Abstract

An expedient and cost-effective diagnostic tool is needed to complement galactography and exfoliative cytology for detection of benign or malignant breast diseases with nipple discharge. The aim of this prospective study is to explore the utility of carcinoembryonic antigen, cancer antigen 15-3 and cancer antigen 125 levels in nipple discharge for the diagnosis of various breast diseases. We evaluated the pre-operative tumor marker levels in 153 nipple discharge samples collected from one or both breasts of 142 women undergoing surgery. Patients with nipple discharge underwent auxiliary examination (ultrasonography, exfoliative cytology, ductoscopy and galactography). Statistically higher levels of carcinoembryonic antigen and cancer antigen 15-3 were found in patients in the malignant group as compared to those in the benign group. No statistically significant difference in the level of cancer antigen 125 ($P = 0.895$). Sensitivities of carcinoembryonic antigen and cancer antigen 15-3 for diagnosing breast cancer were 74.42% and 58.14%, and specificities were 87.27% and 80.00% where as the cutoff values with max-sum of sensitivity and specificity were 224.3 ng/ml and 1368.2 U/ml, respectively. The following sensitivities for telling malignant from benign could be determined: exfoliative cytology 46.67%, ultrasonography 76.74%, galactography 75.00%, and ductoscopy 0%. Exfoliative cytology was found to be a valuable alternative method for differentiating benign from malignancy. Thus, tumor marker analysis of nipple discharge fluid for carcinoembryonic antigen and cancer antigen 15-3 would enhance the accurate assessment and treatment planning for patients with nipple discharge.

Key Words: breast cancer, cancer antigen 15-3, carcinoembryonic antigen, diagnosis, imaging examination, nipple discharge, tumor marker

Introduction

Breast cancer is considered as the most prevalent cancers in Southeast Asian women. Among women in East Asian, breast cancer is second only to gastric cancer. The incidence of breast cancer in some areas of China is increasing by 3-4% per year, greater than that of the worldwide increasing rate (27). Recently, besides breast mass and breast pain, nipple discharge is also a relatively common breast complaint accounting for up to 5% of which women seek medical advice (25). Of the patients presented with nipple discharge,
10-20% would have underlying malignancy (14, 16). Unfortunately, physical examination does not identify a significant number of patients with nipple discharge, and mammography misses 10% to 40% of early breast cancer (9, 24). Galactography and exfoliative cytology are common employed diagnostic tools in the work up of patients with nipple discharge. The percentage of galactography abnormality in patients with nipple discharge is 59.6% (5). Exfoliative cytology was 7% sensitive (18). Both modalities have low sensitivity in the detection of atypia or cancer.

An effective diagnostic tool is needed for accurate stratification of nipple discharge patients for further work up and treatment. Levels of tumor markers in nipple discharge may help to establish the diagnosis of different breast diseases. Tumor markers, such as carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3) and cancer antigen 125 (CA125) are all glycoproteins with altered glycan profiles in cancer progression. CEA was identified in 1965 as the first human tumor associated antigen and serum tumor marker (7). CEA is overexpressed in the majority of colon cancers, half of all breast cancers and nonsmall cell lung cancers (11, 13, 28). CA15-3 is a transmembrane glycoprotein expressed in normal epithelial cells and up-regulated in carcinomas of epithelial origin, including breast cancer, ovarian cancer, pancreatic cancer, and multiple myeloma (23). CA15-3 has been shown to be an independent predictor of cancer recurrence as well as a powerful prognostic indicator for patients at advanced-stage breast cancer (3). CA125 was identified in 1983 as a serum tumor marker for ovarian cancer (2) and was recognized as a membrane-associated mucin found on the apical membrane of epithelial cells of the ocular surface, respiratory tract and female reproductive tract (17). CA125 was found to be up-regulated in breast cancer tissues and not expressed in non-neoplastic ducts (10). However, the abnormal higher level of CA125 is also noticed in sera of some patients with nonmalignant conditions, such as liver disease, peritonitis and pregnant women (21).

Nipple discharge contains concentrated proteins secreted from the ductal and lobular epithelium (20). Analysis of the fluid from discharge would likely shed light on the underlying malignant or benign cause of nipple discharge. No study has explored the utility of CEA, CA15-3 and CA125 level in nipple discharge in the diagnosis of breast diseases in Chinese. The aim of this study is to prospectively evaluate the levels CEA, CA15-3 and CA125 in nipple discharge patients undergoing surgery and correlate the tumor marker levels with the underlying breast conditions. This study also examined the impact of nipple discharge test on the evaluation of abnormal nipple discharge. Their presenting signs as well as ultrasonography, exfoliative cytology, ductoscopy and galactography were evaluated.

Materials and Methods

Subjects

One hundred and fifty-three nipple discharge samples were collected from one or both breasts of 142 women ages 17-76 years old (median = 46) undergoing breast surgery at Qilu Hospital of Shandong University from February 2012 to March 2013. Informed consents were obtained from all participants. This study was approved by ethics committee of Qilu Hospital of Shandong University. The study cohort included women with unilateral or bilateral nipple discharge. There were 11 patients with bilateral nipple discharge. Thus, a total of 153 samples were available for analysis. Samples were classified on the basis of their postoperative pathologic diagnosis. The patients with benign breast diseases included 84 with intraductal papilloma, 10 with mammary duct ectasia, 9 with cyclomastopathy and 7 with breast fibroadenoma. The breast cancer patients included 18 with invasive ductal carcinoma, 15 with ductal carcinoma in situ and 10 with intraductal papillary carcinoma (Fig. 1). The characteristics of patients, ultrasonography, exfoliative cytology, ductoscopy and galactography findings and all pathology reports were recorded by the principal investigator. Ultrasonography was performed on all patients. Exfoliative cytology was performed in 88 patients for 94 lesions. Ductoscopy was performed in 31 patients for 33 lesions. Galactography was performed in 88 patients for 93 lesions.

Nipple Discharge Collection and Laboratory Methods

Nipple discharge samples were collected by a trained surgeon using eppendorf tubes. Nipple was cleansed first with alcohol swabs to remove cellular debris. Nipple discharge was expressed by manual compression of the breast. No serious complications occurred. Droplet of nipple discharge was collected in an eppendorf tube. The tube was then stored in dedicated refrigerator at 4°C. The quantity of collected nipple discharge varied from 20 μL to 200 μL. Samples were transported to the laboratory department within 8 h after collection. Viscous samples were diluted up to 20-fold with normal saline before centrifugation and storage at 4°C. Concentrations of CEA, CA15-3 and CA125 in nipple discharge were measured via an automated test system utilizing sandwich electrochemiluminescence immunoassay (ECLIA) assay kits (Roche cobas e601 analyzer, Roche Diagnostics). All tumor markers assays were performed at Qilu Hospital of Shandong University according to manufacturer’s protocol. The laboratory personnel were blinded to
the clinical information. Commercial reference control sera were used for quality control and calibration.

**Auxiliary Examination**

Exfoliative cytology review was performed on two slides stained using the Papanicolaou method. The slides were examined without the knowledge of clinical or pathologic findings. All slides were evaluated by a single cytopathologist. Each specimen was classified as benign, mildly atypical, severely atypical, and malignant. A positive cytologic test was defined as severely atypical or malignant. Galactography was performed with 0.1 to 0.4 ml of iopromide injected in one single duct. Craniocaudal and 90° lateral views were obtained, as were compression views when appropriate. For the purpose of the study, the galactographic findings were classified into 2 groups: [1] normal and [2] tumor(s). Group 2 includes intraductal filling defects with or without duct dilatation or obstruction. The sonographic findings were classified as [1] normal, [2] tumor(s). Groups 1 was classified as having negative and group 2 as having positive sonographic findings. If ductoscopy revealed significant findings (1 or more papillomatous growths), it was included into positive group. If the ductoscopy findings were normal, or if insignificant “abnormalities” were present (wispy fronds or flat red patches), it was included into negative group.

**Statistical Analysis**

The difference between malignant group and benign group was compared with non-parametric t-test. \( P < 0.05 \) was considered statistically significant. One-way ANOVA (analysis of variance) was performed when comparisons between two groups were made. Chi Square analysis was applied to categorical data (such as ultrasonography, exfoliative cytology, ductoscopy and galactography). Levels of tumor markers are expressed in the form of the mean ± standard deviation. Receiver operating characteristic (ROC) curves were drawn for CEA and CA15-3 based on sensitivity and specificity. ROC analysis was used to identify the cutoff values of different tumor markers. Samples from patients with histologic-proven breast malignancies were used in determining cutoff values with max-sum of sensitivity and specificity. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated between two groups. Accuracy was defined as (true-positive + true-negative) / (true-positive + false-positive + true-negative + false-negative). Pearson’s Chi-square test was used to compare the concordance in both detection methods. The \( P \) value (0.05) of \( \chi^2 \) test was used to determine the difference of rates among tumor markers (CEA and CA15-3) of breast cancer. The combination of tumor markers was carried out in a parallel manner with an “and” rule.
wherein the test result was calculated as positive if the cutoff point for CEA and CA15-3 were both exceeded. Statistical analysis was performed using SPSS software (version 17.0; SPSS, Inc.) and data analysis software (Microsoft Excel 2003).

### Results

One hundred and ten samples were collected from histologically proven benign breast disease and 43 were collected from histologically proven breast malignancies based on postoperative pathologic evaluation (Fig. 1). Mean age for the cohort with benign conditions was 45.43 ± 10.90 years (range 17-72). Mean age for the cohort with malignancy was 49.53 ± 11.62 years (range 31-76). CEA, CA15-3 and CA125 levels ranged from 0.2 ng/ml to 39772 ng/ml (median - 114 ng/ml), 1 U/ml to 6000 U/ml (median- 484.4 U/ml) and 19.73 U/ml to 93800 U/ml (median-16800 U/ml), respectively. Median levels of CEA, CA15-3 and CA125 in nipple discharge exceeded control serum tumor marker levels (0-5 ng/ml, 0-25 U/ml and 0-35 U/ml) by approximately 23-fold, 20-fold and 480-fold, respectively. Fig. 2 shows concentrations of CEA, CA15-3 and CA125 in nipple discharge in malignant group and benign group. There were statistically higher concentrations of CEA and CA15-3 in the malignant group. However, there was no statistically significant difference in the level of CA125 (P = 0.895) between the two groups. Majority of the patients in the benign group were diagnosed with intraductal papilloma (n = 84). No statistically significant difference in CEA, CA15-3 and CA125 levels was found between the intraductal papilloma group and other benign breast disease group (Table 1).

Table 1. Tumor marker levels for intraductal papilloma and other benign breast diseases (mean ± standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Intraductal Papilloma (n = 84)</th>
<th>Other Benign Breast Diseases (n = 26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>97.22 ± 111.43</td>
<td>130.58 ± 152.98</td>
<td>0.227</td>
</tr>
<tr>
<td>CA15-3</td>
<td>946.20 ± 1691.70</td>
<td>1679.73 ± 2515.24</td>
<td>0.173</td>
</tr>
<tr>
<td>CA125</td>
<td>34431.89 ± 41827.64</td>
<td>76586.05 ± 107292.68</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Fig. 2. The CEA, CA15-3 and CA125 levels in nipple discharge: statistically higher concentrations of CEA and CA15-3 in the malignant group (P = 0.008, P = 0.029, respectively). No statistically significant difference in the level of CA125 (P = 0.895) between the two groups.
The combination of tumor markers (CEA and CA15-3) demonstrates that an increase of PPV to 73.33% (Fig. 3). As a candidate tumor marker for breast cancer, the sensitivity and specificity of CEA (cutoff level of 601.4 ng/ml) in nipple discharge was 48.8% and 100%, respectively. For CA15-3 (cutoff level of 9844 U/ml), sensitivity was 11.6% and specificity was 100% (Table 2).

The results of preoperative investigations were compared with the end histological diagnosis obtained from surgery to determine their sensitivity and specificity at predicting malignancy. Table 3 shows the results of ultrasonography, exfoliative cytology, ductoscopy and galactography compared to histological results.

While intraductal papilloma is the most common...
Table 4. Sensitivity and specificity of the predictive factors

<table>
<thead>
<tr>
<th></th>
<th>CEA</th>
<th>CA15-3</th>
<th>Exfoliative Cytology</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>74.42</td>
<td>58.14</td>
<td>46.67</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>87.27</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>69.57</td>
<td>53.19</td>
<td>100</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>89.72</td>
<td>83.02</td>
<td>80</td>
</tr>
</tbody>
</table>

NPV = negative predictive value, PPV = positive predictive value.

caused by nipple discharge, breast cancer is another possible diagnosis. Atypical cytology was common in breasts with nipple discharge than those without (19). Although exfoliative cytology has been used to further evaluate the discharge, it has been associated with a false-negative rate of up to 18% (12, 22). Nipple discharge cytology is specific in cases of malignancy but often inadequate for routine assessment. The accuracies of mammary pump and ductal lavage for breast cancer are 70% and 20%, respectively (4, 30, 31). Ductal brushing samples and ductal lavage fluid provide the main predictive value for the diagnosis of benign diseases (8, 26) not for breast cancer. Ductoscopy and galactography were shown to be accurate in providing the location and depth of ductal abnormalities when a single duct is identified as the source. However, these tests were not to be a useful diagnostic tool for differentiating benign from malignancy. It should be mentioned that despite being poor predictors of malignant pathology in patients with nipple discharge, ultrasound is still vital in the routine work-up of these patients to exclude concomitant pathology in either breast.

Evaluation of serum tumor markers in the diagnosis of breast malignancy has limitations due to low sensitivity and specificity, varies from 19.6% to 54% (1, 6). In breast cancer, tumor markers in serum largely overlap those found in healthy women or women with benign breast disease which not make them as a part of patient management during diagnosis and therapy (6). Measurement of tumor markers in nipple discharge is a simple, low risk, noninvasive and relatively painless preoperative diagnostic tool. The advantage of nipple discharge tumor marker analysis is the presence of concentrated secreted proteins. Thus, trace amounts were sufficient for analysis of target tumor markers. It has been proposed as an alternative way of establishing a diagnosis of breast malignancy. Previous studies investigated to explore CEA as a single tumor marker in nipple discharge which has the lower sensitivity (35.42-48%) (32, 33). There is no literature reported that CA15-3 as a tumor marker in nipple discharge to detect breast cancer. We examined CEA, CA15-3 and CA125 in nipple discharge to determine whether these tumor markers might be useful for breast cancer detection. Based on our data, we recommend the use of CEA and CA15-3 evaluation of nipple discharge. Concentrations of CEA and CA15-3 were significantly higher in samples from malignant group than benign group. CA125 had no clinical significance in differentiating between two groups. No significant difference was observed between levels of CEA and CA15-3 from intraductal papilloma and other benign breast diseases group. The best model of CEA was 74.42% sensitive and 87.27% specific, and that of CA15-3 was 58.14% sensitive and 80.00% specific in the diagnosis of breast malignancies. Combination of CA15-3 and CEA had a greater specificity and PPV than that of CEA or CA15-3 alone but a lower sensitivity, NPV and accuracy than CEA or CA15-3 alone. CA125 levels in nipple discharge were significantly higher than serum levels, but no statistically significant difference between the CA125 levels in malignant group and benign group was found.

Based on immunohistochemistry studies of breast cancer tissues, CEA and CA125 expression in breast cancer were variable and heterogeneous. CEA-reactive cells were seen in 45.2% cases and CA15-3 was aberrantly overexpressed in 37.0% of patients (15, 29). In contrast we have observed significantly higher levels of CEA and CA15-3 in nipple discharge than those of sera and pathological specimen. These observations suggest the evaluation of nipple discharge tumor marker levels can aid in establishing diagnosis of malignant breast diseases, and can help guide surgical planning for patients with nipple discharge. Based on our data, patients with nipple discharge could be safely managed with expectant follow-up if diagnostic examination including galactography, exfoliative cytology, and measurement of tumor markers (CEA and CA15-3) in nipple discharge and serum are all negative. Moreover, if levels of CEA and CA15-3 in nipple discharge are abnormal, surgery should be strongly considered.

There are limitations in our study. Sample size of our study was relatively small. Further study with a larger sample population is underway to validate our findings regarding the diagnostic utility of tumor marker levels in nipple discharge. Furthermore, it was unclear if higher tumor marker concentrations in nipple discharge correlated with greater tumor burden, cancer progression or poor outcomes. Studies with long-term follow-up are needed to address these questions.

Patients with pathological nipple discharge can often be a diagnostic challenge. Current investigations such as ultrasonography, ductoscopy and galactography can be unreliable in this group. Exfoliative cytology has a diagnostic role in patients where such an investigation showed the low sensitivity of malignancy. In summary, this study demonstrated the feasibility of evaluating tumor markers in nipple discharge fluid.
CEA and CA15-3 levels in nipple discharge can aid in the preoperative confirmation of breast cancer.

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References


