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Original Article

Levels of CEA, CA153, CA199, CA724 and AFP in nipple discharge of breast cancer patients

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Abstract: The distinction between breast cancer and benign breast diseases with nipple discharge remains an important diagnostic challenge. The purpose of this study was to predict the potential usefulness of tumor markers in nipple discharge and to investigate the relationship of tumor markers and clinical characteristics with breast cancer. One hundred and eleven patients with nipple discharge received breast surgery from November 2013 to December 2014 were included in the study. We evaluated levels of five tumor markers (CEA, CA153, CA199, CA724 and AFP) prior to treatment. Patients were divided into two groups according to postoperative pathological results: 30 cases in breast cancer group and 81 cases in benign group. The relationships of clinical characteristics with breast cancer were investigated by multivariate analysis with a logistic regression model.It showed significant differences in levels of nipple discharge CEA ($P < 0.001$) and CA153 ($P = 0.014$), but not CA199 ($P = 0.856$), CA724 ($P = 0.171$), AFP ($P = 0.834$) among two groups. Logistic regression analysis demonstrated complaint, age, menopause, abnormal palpable mass, CEA and CA153 were associated with breast cancer. In summary, measurements of CA199, CA724 and AFP in nipple discharge are not of great clinical value. Detecting CEA and CA153 in nipple dischargecould potentially be used for the early detection of breast cancer with in high-risk populations.

Keywords: Breast cancer, diagnosis, nipple discharge, CA199, CA724, AFP

Introduction

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 [1]. Of the patients presented with nipple discharge, 10-20% would have underlying malignancy [2, 3].

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 153 (CA-153), cancer antigen 199 (CA199), cancer antigen 724 (CA724) and alpha-fetoprotein (AFP) are all glycoproteins with altered glycan profiles in cancer progression. CEA is overexpressed in the majority of colon cancers, half of all breast cancers and nonsmall cell lung cancers [4-6]. CA153 is a transmembrane glycoprotein up-regulated in carcinomas of epithelial origin, including breast cancer, ovarian cancer, pancreatic cancer, and multiple myeloma [7]. CA-153 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 [8]. In our previous study, preoperative CEA, CA153 independently indicated reliability and predictability in breast cancer with nipple discharge. Patients with CEA > 224.3 ng/mL and CA153 > 1368.2 U/mL generally had a breast cancer pathological finding.It was clarified thatCA199 and AFP had increased serum levels in human breast cancer cases [9, 10]. AFP was considered as a breast cancer risk factor [11, 12]. CA724 was expressed in colorectal, gastric cancer and ovarian cancer [13]. However, CA724, CEA and CA199 combination was considerable to improve sensitivity without impairing specificity [13]. Bian also indicated that combined assay of CA724 and CA153 can provide diagnosis value for ovarian cancer [14, 15]. According to

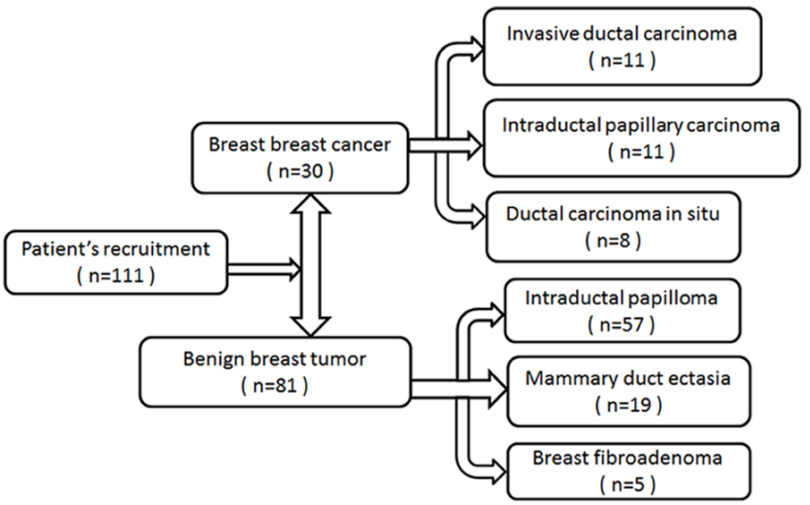

Figure 1. Patientsrecruitment.

previous studies, combination of CA724 and other tumor markers may improve diagnosis for clinical use.

In this study, we determined levels of five tumor markers in nipple discharge and verify CEA and CA153 diagnostic values in differentiation of breast cancer and benign breast diseases. The present study was also aimed to find out the potential useful tumor markers including CA199, CA724 and AFP to use as supplementary test for the diagnosis of breast cancer. At the same time, we evaluated the relationship between tumor markers, other clinical characteristics like age, menopausal status in women with nipple discharge and breast cancer.

Materials and methods

Subject

A total of 111 patients with complaint of nipple discharge were scheduled to have surgeries were included in our study between November 2013 and December 2014 in Qilu Hospital of Shandong University. 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 nipple discharge. Patients had received no preoperative treatment. They were divided into two groups (breast cancer group and benign group) according to the postoperative pathological diagnosis. 111 patients were pathologically confirmed postoperatively with breast cancer, including the following histological subtypes: invasive ductal carcinoma (n = 11), intraductal papillary carcinoma (n = 11), ductal carcinoma in situ (n = 8). 30 patients were diagnosed with benign breast diseases including intraductal papilloma (n = 57), mammary duct ectasia (n = 19), breast fibroadenoma (n = 5) (**Figure 1**).

Nipple discharge collection and laboratory methods

All samples were collect ed before any treatment was initiated within 2 days after hospitalization. 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 hours after collection. Viscous samples were diluted up to 20-fold with normal saline before centrifugation and storage at 4°C. Concentrations of CEA, CA153, CA199, CA724 and AFP in nipple discharge were quantitativelymeasured *via* an automated test system utilizing sandwich electro chemiluminescence 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.

Statistical analysis

Data of tumor markers were expressed as mean ± standard error of the mean (SEM). Comparisons of data between two groups were performed using Kruskal-Wallis test or Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was used to identify the cut-off values of CEA and CA153. Samples from

Table 1. Logistic regression results for the relationship between breast cancer and benign breast diseases

Variable	Subgroup	Breast cancer	Benign breast diseases	Univariate analysis		Multivariate analysis	
		Total no.	Total no.	OR (95% CI.)	*P*	OR (95% CI.)	*P*
Complaint				3.66 (1.45-9.22)	0.006**	0.66 (0.17-2.65)	0.561
	Nipple discharge	17	67				
	Mass with nipple discharge	13	14				
Side of nipple discharge						0.125	
	Left	15	25	2.05 (0.52-8.17)	0.307		
	Right	12	37	0.54 (0.22-1.35)	0.187		
	Both	3	19				
Age				4.19 (1.67-10.51)	0.002**	12.68 (1.11-144.47)	0.041*
	< 50	16	67				
	≥ 50	14	14				
Menarche				1.06 (0.45-2.46)		0.898	
	≤ 14	17	47				
	> 14	13	34				
Menopause				4.87 (1.86-12.74)	0.001**	0.87 (0.09-8.65)	0.903
	Premenopausal	17	70				
	Postmenopausal	13	11				
Average menstrual cycle				0.87 (0.38-2.02)		0.745	
	≤ 28	16	46				
	> 28	14	35				
Age at first child						0.587	
	≤ 26	13	44	1.45 (0.33-6.42)	0.624		
	≤ 30	3	7	0.92 (0.21-4.09)	0.911		
	> 30	14	30				
Number of child				2.74 (0.32-23.29)		0.355	
	Nullipara	1	7				
	Multipara	29	74				
Lactation				1.53 (0.31-7.67)		0.602	
	No	2	8				
	Yes	28	73				
Interval between menarche and primiparity				1.14 (0.41-3.22)		0.801	
	< 10	24	63				
	≥ 10	6	18				
Family history				0.96 (0.31-2.93)		0.94	
	Yes	5	14				
	No	25	67				
Mammary gland disease history				0.25 (0.03-2.00)		0.189	
	Yes	1	10				
	No	29	71				
BMI						0.497	
	< 25	7	27	2.41 (0.14-40.26)	0.541		
	< 30	22	53	3.86 (0.21-69.67)	0.361		
	≥ 30	1	1				
Palpable mass				4.1 (1.70-9.92)	0.002**	5.72 (1.48-22.18)	0.012*
	No	11	57				
	Yes	19	24				
CEA level				6.60 (2.63-16.57)	< 0.001**	8.51 (2.60-27.86)	< 0.001**
	< 224.3 ng/ml	12	66				
	≥ 224.3 ng/ml	18	15				
CA153 level				2.88 (1.17-7.07)	0.021*	4.88 (1.37-17.36)	0.015*
	< 1368.2 U/ml	17	64				
	≥ 1368.2 U/ml	13	17				

Complaint, age, menopause, palpable mass, CEA level and CA153 level showed significant differences between the two groups. Abbreviation: 95% CI, 95% confidence interval; BMI, Body index. *$P < 0.05$, **$P < 0.01$.

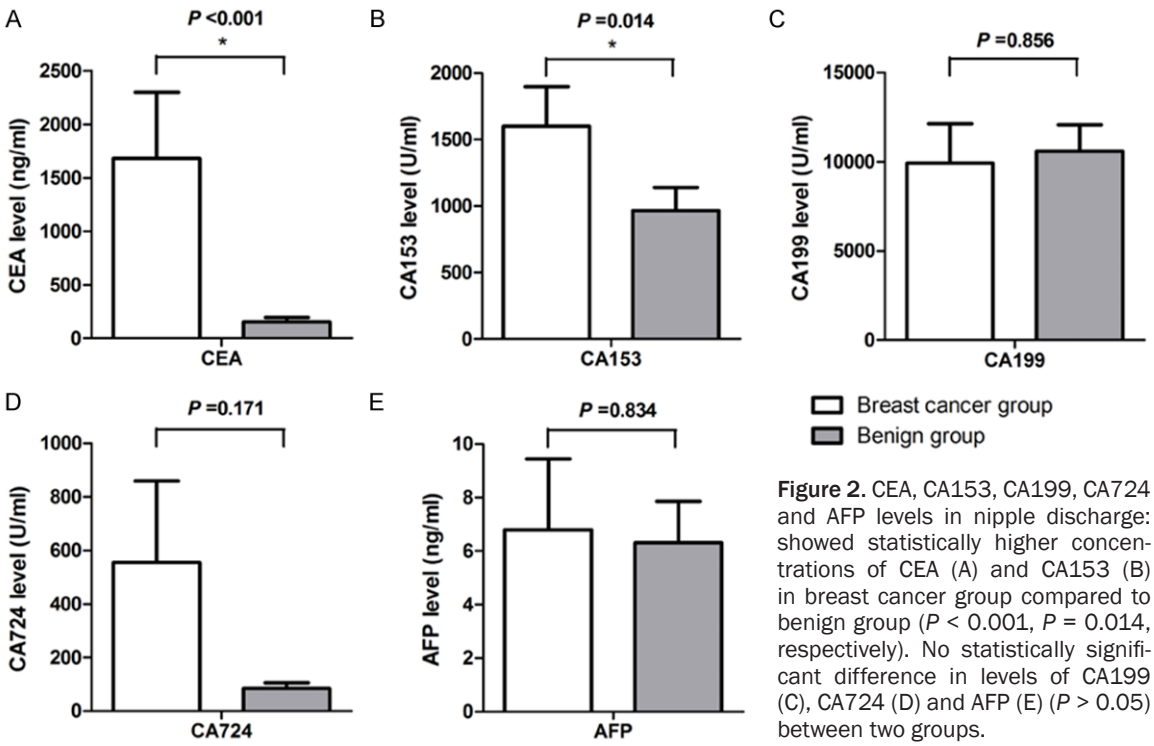

Figure 2. CEA, CA153, CA199, CA724 and AFP levels in nipple discharge: showed statistically higher concentrations of CEA (A) and CA153 (B) in breast cancer group compared to benign group ($P < 0.001$, $P = 0.014$, respectively). No statistically significant difference in levels of CA199 (C), CA724 (D) and AFP (E) ($P > 0.05$) between two groups.

patients with histologic-proven breast malignancies were used in determining cutoff values with max-sum of sensitivity and specificity. Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated based on the cutoff value for CEA and CA153. Patients' clinical characteristics were summarized descriptively. Detailed clinical characteristics of patients including complaint, side of nipple discharge, age, menarche, menopause, average menstrual cycle, age at first child, number of child, lactation, Interval between menarche and primiparity, family history, mammary gland disease history, body mass index and palpable mass-were obtained to build electronic databases. The Pearson Chi-square or Fisher's exact test was used to evaluate these qualitative data. Logistic regression analysis was used to select the risk factors for breast cancer probability. Variables that achieved statistical significance in univariate analysis were included subsequently in a multivariate analysis. All P values were two-sided, $P < 0.05$ was considered statistically significant. Statistical analysis was carried out using SPSS 17.0 software.

Results

There are 111 patients enrolled in the study hospitalized for breast surgery. The patients are aged between 20 and 72 years old (average = 44.7). It showed that invasive ductal carcinoma (n = 11, 36.7%) and intraductal papillary carcinoma (n = 11, 36.7%) were the main cause of breast cancer with nipple discharge, followed by ductal carcinoma in situ (n = 8, 26.7%). It showed that intraductal papilloma (n = 57, 70.4%) was the main cause of benign diseases with nipple discharge (**Figure 1**). The clinical characteristics of the patients are summarized in **Table 1**. The mean ages of the two groups were 48.4 years (range, 28-72 years) in breast cancer group and 43.3 years (range, 20-64 years) in benign group, respectively, and statistic significance differences can be found ($P = 0.002$). Complaints, menopausal age, palpable mass in preoperative examination were compared between the two groups, statistic significant differences ($P < 0.05$) were observed. There was no statistical difference ($P > 0.05$) between two groups in term of general clinical data, such as side of nipple discharge, menarche, age at first child, etc.

The levels of CEA, CA153, CA199, CA724 and AFP in nipple discharge of patients in two groups are presented in **Figure 2**. Mann-Whitney U test for differences between the studied groups in nipple discharge tumor markers were performed. The levels of CEA and CA153 in the breast cancer group were signifi-

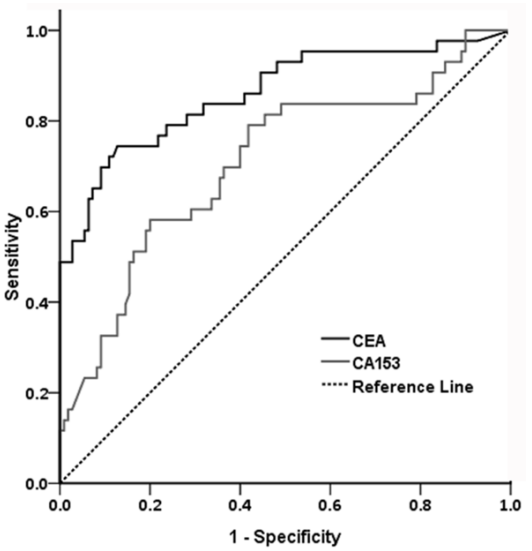

Figure 3. Receiver operating characteristic (ROC) analysis for the diagnosis of CEA and CA153 in nipple discharge. According to the analysis in previous study (cancerous breasts = 43, benign controls = 110), cut-off values of CEA and CA153 were assessed (CEA was 224.3 ng/ml and CA153 was 1368.2 U/ml, respectively).

cantly higher than those of the benign group ($P < 0.05$). The capacity of CEA and CA153 to differentiate causes of nipple discharge was assessed with cutoff values made by previous study. Evaluation of ROC analysis based on sensitivity and specificity established cut-off values for CEA and CA153 (**Figure 3**). The cut-off value of CEA was 224.3 ng/ml and CA153 was 1368.2 U/ml, respectively. Levels of the other three tumor markers (CA199, CA724 and AFP) newly added in the study had no statistical difference between two groups ($P > 0.05$).

When used to analyze breast cancer group versus benign group, the sensitivities, specificities, positive predictive value (PPV) and negative predictive value (NPV) of CEA and CA153 at their respective cut-off values are outlined in **Table 2**. In this situation, we calculated that sensitivity was 60.0%, specificity was 81.48%, positive predictive value was 54.55%, negative predictive value 84.62%, and accuracy was 75.68% for CEA which performed better than CA153.

In **Table 1**, breast cancer were more likely in older patients (age > 50) than younger woman ($P = 0.001$). It was observed that patients with the complaint of masses with nipple discharge would probably be diagnosed as breast cancer ($P = 0.006$). Postmenopausal women were significantly different from premenopausal women in the breast cancer group ($P = 0.001$). In addition, patients with breast cancer showed a significant correlation with palpable mass in physical examination ($P = 0.002$). Combining with significant values of CEA and CA153, a multiple logistic regression analysis revealed that breast cancer was significantly related to four factors: age, palpable mass, CEA level and CA153 level. These four factors were used as a dependent variable associated with breast cancer.

Discussion

Currently, there are few studies on nipple discharge tumor markers in detection of breast cancer.In our previous study, the identification of CEA and CA153 for detection of breast cancer with nipple discharge has produced given definite indications for their possible use in clinical practice. In this study, we investigated CA199, CA724 and AFP as potential tumor markers and validated CEA and CA153 to differentiate breast cancer from benign diseases.

The presence of tumor markers, such as CEA and CA199 is closely associated with invasion and metastasis of many malignancies [16]. CA199 is of great clinical value for the diagnosis and prognosis of pancreatic cancer [17], and also had been observed in gastrointestinal cancer [18-20]. Other potential markers are CA724, known for colorectal and gastric cancer, ovarian cancer [21, 22], AFP also known for ovary, testis and liver cancer [23]. A previous study indicated that AFP in serum suppressed estrogen-dependent growth of breast cancer cells [24]. In other two studies, it reported protective associations of maternal breast cancer with increasing circulating levels of AFP, particularly at younger ages [25, 26]. Unfortunately, there is paucity of article showed the level of CA199, CA724 and AFP had relationship to breast cancer with nipple discharge in the past.

Our results showed that the levels of CEA and CA153 in breast cancer group were significantly higher than those of the control group ($P < 0.05$). It obtained coincide with the previous experimental results. It indicated that CEA and CA153 can play an important role in the process of diagnosis in breast cancer with nipple

Table 2. Diagnostic performance of tumor marker concentrations

Tumor makers	Sensitivity	Specificity	PPV	NPV	Accuracy	Cutoff value
CEA	60.00 (18/30)	81.48 (66/81)	54.55 (18/33)	84.62 (66/78)	75.68 (84/111)	224.3
CA153	43.33 (13/30)	80.00 (64/80)	44.83 (13/29)	79.01 (64/81)	70.00 (77/110)	1368.2

Note: Data are percentages, with numbers used to calculate them in parentheses. NPV = negative predictive value, PPV = positive predictive value.

discharge. The present study was the first time to predict the potential usefulness of CA199, CA724 and AFP in nipple discharge. According to data, mean levels of CA724 in cancer group were higher than benign control but showed no difference between two groups. Nonetheless, CA199 and AFP in cancer group had no statistical significance when compared with benign group. Measurements of CA199, CA724 and AFP in nipple discharge were not of great clinical value for assessment to the clinician.

This study evaluated the correlations of significant tumor markers and patient clinical characteristics with postoperative pathological analysis. Moreover, Chi-square test showed complaint, age, menopause status, palpable mass in physical examination, CEA and CA153 were all strongly associated with breast cancer. These results showed that above factors were correlated with the occurrence and development of breast cancer. The patients with breast cancer exhibited significantly higher CEA and CA153 than those with benign breast diseases. We also showed that patients in cancer group were significantly older than controls. Masses in complaints or physical examination and postmenopausal status occurred more frequently in breast cancer patients. Nonetheless, themultivariate logistic regression analysis revealed age, palpable mass in physical examination, CEA and CA153 showed independent influential factors for breast cancer, differences between complaint and menopause status did not reach statistical significance.

When it comes to the limitations of this study, because the cases only came from the Department of Breast Surgery, Qilu Hospital of Shandong University, a small number of cases may influence the representativeness of two groups. Further researches including multi-center studies are still necessary for better understanding the relationship between multiple clinical factors and breast cancer with nipple discharge. Nonetheless, not all patients with nipple discharge had all five tumor markers analyzed at one time, due primarily to the identification of enrolled markers at various time points during the study. We didn't have a follow-up of the patient's prognosis and survival rates. So whether CEA and CA153 could be used as prognostic factors of breast cancer is to be discussed. Further investigation on the correlation between screening, disease progress, follow-up monitoring and treatment response is required.

In conclusion, based on data in the present study, tumor markers including CEA and CA153 may be the efficient, cost-effective diagnostic method in breast cancer. It is unreliable to use CA199, CA724 and AFP to predict breast cancer. In general, it is suggested that the combination of CEA, CA153, age and palpable masses in physical examination is of clinical value for distinguishing between breast cancer and other benign diseases. As a potential routine preoperative examination, detection of CEA and CA153 in nipple discharge may improve clinical decisions and play important clinical roles in the early diagnosis, treatment of breast cancer.

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Disclosure of conflict of interest

None.

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