Original Article

Utility of serum total and free prostate-specific antigen in combination with serum carbohydrate antigen 15-3 and carcinoembryonic antigen in breast tumours

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Abstract

Background: Prostate Specific Antigen (PSA) is secreted by prostate gland as well as hormonally regulated tissues such as breast, ovaries and endometrium. We aimed to assess the utility of serum total and free PSA in combination with carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in diagnosis of breast cancer.

Methods: Seventy two female patients (38 with benign breast disease and 34 with malignant breast disease) who were histologically, cytologically confirmed with diagnosis of primary breast tumours were investigated. Serum total prostate specific antigen (PSA), Free PSA, CEA, CA 15-3 were analysed by enzyme linked immunosorbent assay (ELISA) method. Diagnostic performance of markers was studied using receiver operating characteristic curve and logistic regression analysis. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Results: Patients with malignant breast cancer had significantly higher levels of all tumour markers compared to benign breast tumours. A significant decrease in total PSA, CEA and a statistically insignificant decrease in free PSA concentrations were seen in malignant breast cancer patients after surgery. Performance of total PSA was best among all the markers with 100% sensitivity, NPV, 94.7% specificity and 94.4% PPV.

Conclusions: Serum total PSA is a good diagnostic marker to differentiate benign breast disease from malignant tumours compared to currently used CEA and CA 15-3.

Keywords: Breast cancer, carbohydrate antigen 15-3, carcinoembryonic antigen, prostate-specific antigen, tumour markers

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INTRODUCTION

Breast tumours are one of the leading causes of morbidity and mortality among the various types of cancers affecting women globally. In India, breast cancer is the second most common cancer diagnosed in women after cervical cancer, and the incidence is found to be rising. In 2008, it was estimated that there were 115,251 new cases of breast cancer with an age standardised

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incidence rate of 22.9/100,000. By 2030, it is estimated that the number of new cases of breast cancer in India will reach just under 2,00,000 per annum.[1] Various screening and diagnostic modalities are available for the detection of breast cancer. 'Triple test' is one of the common accepted diagnostic procedures for identifying breast tumours having its own limitations; and hence, the search for new diagnostic interventions is needed.[2] Diagnosis by detecting specific breast-derived products in blood may be an easier way to diagnose. In this context, the role of tumour markers in the diagnosis of breast tumours is of special interest. Among these, the most widely used serum tumour markers in breast cancer are carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3).[3]

CEA is a glycoprotein expressed at higher concentrations in tumours of epithelial origin such as carcinoma of colorectum, breast and ovaries and in non-malignant conditions such as gastritis and cirrhosis. CA 15-3 is a mucin expressed at higher levels in conditions such as carcinoma of the breast, ovary and endometrium. Concomitant determination of serum CA 15-3 and CEA in breast cancer increase the sensitivity and reduce the number of false-negative determinations.[4] Because of the ease of serum sample availability, new serum markers to diagnose breast tumours are being studied, of which serum prostate-specific antigen (PSA) is one.

PSA is a glycoprotein that was believed to be produced by epithelial cells of the prostate gland. Later on, studies inferred that PSA is not prostate-specific and is also secreted by hormonally regulated tissues such as breast, ovaries and endometrium.[5,6] PSA exists in free and bound forms. Because of its very low concentrations in females than males, the diagnostic utility of serum PSA in breast cancer was restricted previously.[7] However, a significant improvement in detecting serum PSA using ultrasensitive diagnostic methods has lead to observation of significant higher concentrations in breast neoplasms. Bound form of PSA predominates in normal females, and benign breast conditions in contrast to serum-free PSA in breast cancer.[8] Hence, the present study was carried out to evaluate the utility of serum total PSA and free PSA in combination with serum CA 15-3 and serum CEA to detect breast tumours.

MATERIAL AND METHODS

Study subjects

We investigated 72 female patients comprising 38 patients with benign breast disease and 34 patients with malignant breast disease between September 2013 and August 2014 at a tertiary care hospital in south India after obtaining the institutional ethical clearance and written informed consent. All the patients were histologically and/or cytologically confirmed with the diagnosis of primary breast tumours. We excluded pregnant women, patients having the past or present history of any other gynaecological or other malignancies, undergoing treatment for breast tumours, receiving oral contraceptive pills, hormone replacement therapy, steroid medications and those not willing to participate from the present study.

Other relevant information, including estrogen-receptor -progesterone receptor (ER/PR) status, was obtained from medical records of the patients.

Sample collection

After an overnight fast, 5 mL of venous blood was collected in plain tubes from all the participants. The samples were centrifuged, and the serum was separated and stored at 80°C until further analysis. Second blood sample was collected 1 week after surgery.

Tumour markers, including serum CEA and serum CA 15-3 were analysed by an enzyme-linked immunosorbent assay (ELISA) method (DSI S. r. l, Italy). Serum total and free PSA were analysed by the ELISA method (NovaTec Immunodiagnostica GmbH, Germany). All the tumour markers were analysed on the Chemwell autoanalyzer system.

Statistical analysis

The Kolmogorov–Smirnov test was performed to test the normality of data distribution. Data obtained is expressed as the mean ± standard deviation for continuous variables and as frequency (number [%]) for categorical variables. The difference in all biochemical parameters studied among the study groups was tested using the Independent samples *t*-test, and among carcinoma of breast patients before and after surgery was tested using paired samples *t*-test. The diagnostic performance of the markers studied was studied using the receiver operating characteristic (ROC) curve analysis. The diagnostic relevance of these markers was then assessed using logistic regression analysis. Further, sensitivity, specificity, positive-predictive value (PPV) and negative-predictive values (NPV) were calculated using different cut-off values. Data analysis was performed using the Microsoft Excel spreadsheets, SPSS for windows version 11.5 program (SPSS Inc., Chicago, IL, USA) and Medcalc statistical software (version 13.2.2, Belgium). A value of $P < 0.05$ was considered as statistically significant.

RESULTS

The demographic profile of patients with benign and malignant tumours enrolled in this study is described in Table 1. Patients with malignant breast tumours were significantly older (50.1 ± 10.3 vs. 36.9 ± 10.8 years; $P < 0.0001$) attained menarche at later age (14.1 ± 0.8 vs. 13.6 ± 0.8 years; $P = 0.0002$) and had a greater body mass index 24.2 ± 4.3 vs. 21.4 ± 4.6 kg/m^2; $P = 0.009$) compared to patients diagnosed with a benign breast tumour. A family history of breast cancer was observed in 10.5% and 20.6% in patients with benign and malignant breast tumours, respectively. ER and PR positivity was observed in 35.3% and 44.1% in patients with malignant breast tumours, respectively.

As shown in Table 2, patients with malignant breast tumours had significantly higher levels of all the tumour markers before surgery compared to the patients with benign breast tumours. A significant decrease in serum total PSA and serum CEA levels was seen in patients with malignant breast tumours after surgery.

ROC analysis showed that serum total PSA [Figure 1a] had a greater area under the curve (AUC) compared to serum CEA [Figure 1b] and serum CA 15-3 [Figure 1c], suggesting it to have a better diagnostic utility compared to serum CEA and serum CA 15-3. Logistic regression analysis [Tables 3 and 4] showed only serum CA 15-3 was significantly associated with breast cancer with an odd's ratio of 1.059. The combination of serum total PSA and serum CEA was found to perform better than combining serum total PSA with serum CA 15-3 or the combination of all the three markers ($P < 0.0001$) [Table 5]. Since the marker positivity for serum total PSA in patients with malignant breast disease was all above the mean value of the benign group as well as the ROC derived cut-off value (100%), logistic regression analysis for serum TPSA was not performed.

Marker positivity in patients with benign and malignant tumours

The performance of serum total PSA was the best among all the markers with 100% sensitivity and NPV, 94.7% specificity and 94.4% PPV. There was an improvement in the specificity (77.8% vs. 84.2%), PPV (62.2% vs. 84.8%) and NPV (77.8% vs. 84.6%) for serum CEA with ROC cut-off values compared to cut-off values provided by the manufacturer.

DISCUSSION

In the present study, serum CA 15-3 and serum CEA were significantly higher in malignant breast tumours group

Table 1: Demographic profile of patients with benign and malignant tumours

Parameter	Benign No. (%)	Malignant No. (%)
Age (years)		
15-45	28 (73.7)	15 (44.1)
>45	10 (26.3)	19 (55.9)
BMI (kg/m^2)		
<18.9	11 (28.9)	4 (11.7)
19.0-24.9	21 (55.3)	14 (41.2)
25.0-26.9	5 (13.2)	10 (29.4)
Age at menarche (years)		
<12	2 (5.3)	1 (2.9)
12-14	35 (92.1)	23 (67.6)
>14	1 (2.6)	10 (29.4)
Age at menopause (years)		
≤45	8 (66.6)	10 (55.5)
>45	4 (33.3)	8 (44.5)
ER/PR status		
ER +	-	12 (35.3)
PR +	-	15 (44.1)
Parity		
Nulliparous	3 (9.1)	3 (8.8)
Multiparous	30 (90.9)	31 (91.2)
Family history of breast cancer		
Present	4 (10.5)	7 (20.6)
Absent	34 (89.5)	27 (79.4)

BMI=Body mass index; ER=Estrogen receptor; PR=Progesterone receptor

Table 2: Serum tumour markers in patients with benign and malignant breast tumours

Parameter	Benign	Malignant	
		Before surgery	After surgery
TPSA (ng/mL)	0.14±0.10	0.93±0.47*	0.30±0.22†
FPSA (ng/mL)	0.014±0.012	0.039±0.025*	0.029±0.025
CEA (ng/mL)	4.47±1.62	7.39±1.78*	3.23±1.30†
CA 15-3 (U/mL)	17.31±9.99	28.50±18.83*	28.79±14.42

Data are presented as mean±SD.
*Statistically significant ($P<0.05$) between controls and malignant cases (before surgery); †Statistically significant between malignant cases (before surgery) and after surgery. TPSA=Total prostate-specific antigen; FPSA=Free prostate-specific antigen; CEA=Carcinoembryonic antigen; CA 15-3=Carbohydrate antigen 15-3; SD=Standard deviation

Table 3: Logistic regression analysis

	Serum CEA	Serum CA 15-3
Cut-off	5.4	28.29
OR (95% CI)	1.001 (0.97-1.03)	1.059 (1.016-1.104)
P - value	0.9673	0.0063

CEA=Carcinoembryonic antigen; CA 15-3=Carbohydrate antigen 15-3; CI=Confidence interval; OR=Odd's ratio

Table 4: Logistic regression analysis using combination of markers

	SerumTPSA + serum CA 15-3	SerumTPSA + serum CEA	SerumTPSA + serum CEA + serum CA 15-3
OR (95% CI)	6.82 (2.42-19.20)	50.60 (6.24-410.37)	9.00 (3.08-26.33)
P - value	0.0003	0.0002	0.0001

TPSA=Total prostate-specific antigen; CEA=Carcinoembryonic antigen; CA 15-3=Carbohydrate antigen 15-3; CI=Confidence intervals; OR=Odd's ratio

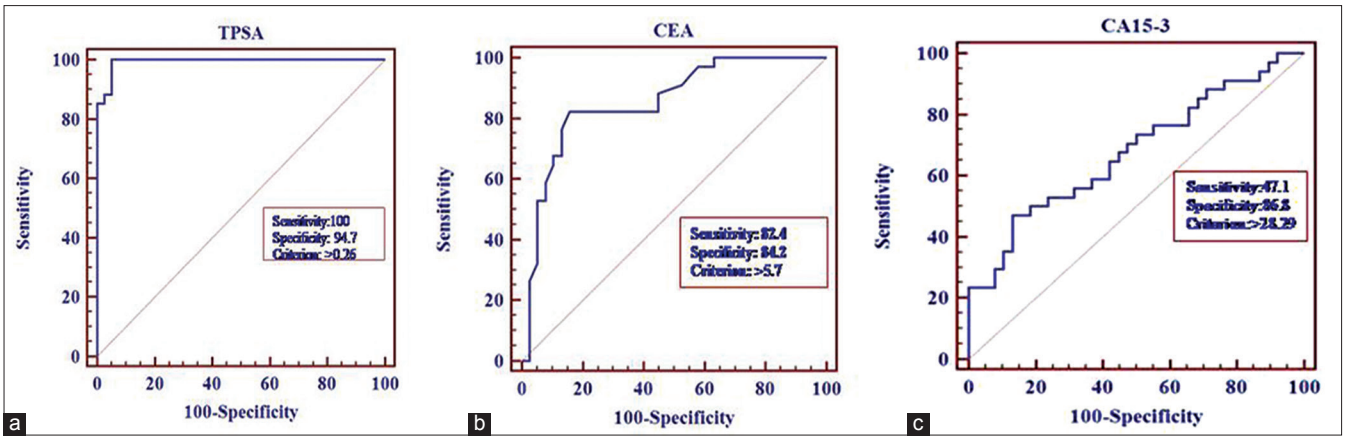

Figure 1: Rece'iver operating characteristic curve analyis. TPSA = Total prostate-specific antigen; CEA = Carcinoembryonic antigen; CA 15-3 = Carbohydrate antigen 15-3

when compared with benign breast tumours group. This is in agreement with previous studies.[9,10]

CA 15-3 is breast cancer-associated antigen expressed by monoclonal antibodies DF_3 found on the surface of human mammary cancer cells and 115D8 antigens found on the milk fat globule membrane. The elevated levels of serum CA 15-3 in malignant breast cases may be due to the over expression of the MUCI gene which encodes serum CA 15-3.[11] The pathophysiological mechanism for elevated serum CEA in the malignant breast tumour group is not exactly known.

Following surgery in patients with malignant breast tumours, serum CEA decreased significantly compared to levels before surgery. This is in agreement with previous studies.[12,13]

However, serum CA 15-3 did not decrease after surgery in the present study. Few studies have reported a significant decrease in serum CA 15-3 after surgery which are contradictory to the findings of the present study.[10,11] Serum CA15-3 has a longer half-life which leads to slower clearance from blood.[10] The possible explanation for persistent elevated serum CA15-3 after surgery in patients with malignancy in the present study could be the time point studied after surgery. The time point of 7 days for sample collection after surgery was taken because of the relative half-lives of CEA, i.e. 3–11 days.,[14] PSA, i.e. 1.5–3.2 days.[15]

Serum total PSA and free PSA levels were significantly higher in patients diagnosed with malignant breast tumours when compared with benign breast tumours. This is in agreement with previous studies.[16,17]

Increase in serum total PSA in women with breast cancer, benign breast disease or uterine fibroids has been proposed

Table 5: Marker positivity for serum TPSA, CEA and CA15-3 in benign and malignant breast tumours based on manufacturer provided cut-off values and the ROC curve cut-off values

Tumour marker	Marker positivity			
	Manufacturer cut-off		ROC cut-off	
	Benign	Malignant	Benign	Malignant
TPSA*	-	-	2 (5.26)	34 (100)
CEA†	17 (44.7)	28 (82.4)	5 (13.16)	28 (73.68)
CA 15-3‡	3 (7.9)	9 (26.5)	6 (15.79)	16 (47.06)

Manufacturer provided cut-off values for *CEA >5.0 ng/mL; †CA 15-3 >35 U/mL. ROC cut-off values for *TPSA >0.26 ng/mL; †CEA >5.7 ng/mL; ‡CA 15-3 >28.29 U/mL.
TPSA=Total prostate-specific antigen; CEA=Carcinoembryonic antigen; CA 15-3=Carbohydrate antigen 15-3; ROC=Receiver operating characteristic

to be the result of a disrupted hormonal balance which triggers the aberrant expression of hormone-dependent genes such as PSA. Mutations in the promoter region of this gene may also cause aberrant expression of PSA and is the cause for high levels of PSA. Breast tissue as a source of serum total PSA is supported by the finding of a significant decrease in its levels in patients with malignant breast tumours after surgery in the present study, which is in agreement with previous studies.[16,18]

On the other hand, a statistically insignificant decrease in serum-free PSA concentrations was seen in patients with malignant breast tumour after surgery when compared to levels before surgery. Previous studies have reported a significant decrease in serum-free PSA levels in patients diagnosed with malignant breast tumours after surgery when compared to levels before surgery, suggesting that this fraction is produced by tumour.[17-19]

Under normal conditions in males, <30% of PSA is in the free form, and the remaining exists in the bound form. Free PSA has been shown to be the predominant molecular form unique to breast carcinoma (>50% of total PSA),

whereas total serum PSA is the predominant molecular form in women with fibro adenomas and other benign breast diseases.

It has been proposed that the tumour produces free PSA that is incapable of binding to alpha-1-antichymotrypsin (ACT) or tumours produce an endopeptidase which causes a post-translational modification of PSA produced by the breast, preventing complex formation with ACT and increasing the proportion of free PSA. However, in the present study, serum total PSA was the predominant molecular form in patients with malignant breast tumours, as is seen in patients with malignant prostate tumours.

The commercial kit employed in the current study had a detection limit of 0.05 ng/mL or 50 ng/L. Only thirteen patients with malignant breast tumours and only one with benign breast tumours had serum-free PSA levels above the detectable range of 0.05 ng/mL. The calculated fraction of serum-free to total PSA in patients with malignant breast tumours was lower compared to what has been reported in the literature. Majority of the patients in the present study had <10% serum-free PSA while only three had >20% serum-free PSA with only one patient having 30% serum free PSA. This needs to be studied further. Hence, serum-free PSA was not considered for further analysis.

The findings of the present study suggest that serum CEA and serum total PSA can be useful as an indicator of adequate surgery and its reappearance can indicate recurrence of breast cancer. Serum total PSA had a greater AUC (AUC = 0.913) compared to serum CEA and serum CA 15.3 (AUC = 0.866 and 0.677, respectively) as shown in Figure 1, suggesting it to have a better diagnostic utility compared to serum CEA and serum CA 15-3. The diagnostic relevance of these markers was then assessed using logistic regression analysis. Logistic regression analysis showed only serum CA 15-3 was significantly associated with breast cancer with an odds ratio of 1.059 (95% confidence interval 1.016–1.104; $P = 0.0063$).

Since the marker positivity for serum TPSA in patients with malignant breast disease was all above the mean value of the benign group as well as the ROC derived cut-off value (100%), logistic regression analysis for serum TPSA was not performed. The 100% positivity obtained in the present study denotes its high discriminatory power to differentiate benign cases from malignant breast tumours. In the present study, combination of serum total PSA and serum CEA was found to perform better than combining serum total PSA with serum CA 15.3 or the combination of all the three markers.

The findings of marker positivity based on cut-off values provided by the manufacturer and those obtained based on ROC derived cut-off values show the importance of using an appropriate cut-off value. An ideal situation would; however, have been based on ROC derived cut-off values with inclusion of a control group. However, over results still hold good since these cut-off values differentiate benign from malignant breast tumours. The marker positivity was best for serum total PSA which further supports its use for diagnosing breast tumours, in the absence of other non-prostatic sources for elevation.

The findings of the present study suggest the usefulness of serum total PSA as a diagnostic marker to differentiate benign breast disease from malignant tumours compared to the currently used markers serum CEA and serum CA 15-3 which exhibit lesser sensitivity and specificity. Serum-free PSA was not found to be useful as has been suggested by previous studies. This could probably be due to smaller sample size, and hence, these findings should be confirmed in a study with larger sample size.

In the presents study control group was not included. Because of lack of available data on normal range for serum total PSA and serum free PSA in females; further work is required to establish the same.

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Conflicts of interest

There are no conflicts of interest.

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