Serum total PSA and free PSA in breast tumors

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Abstract Now a days measurement of molecular forms of PSA has gained importance in clinical practice. Several studies have demonstrated the production of PSA in female tissues, such as breast. The present piece of work has been undertaken with an objective to estimate the relative proportion of the molecular forms of PSA in serum along with serum testosterone in benign and malignant breast tumor cases and to analyze their association with the severity of the disease process 34 malignant and 26 benign breast disease cases along with 33 healthy controls of same age group were enrolled in this study for evaluation. Serum testosterone was measured by ELISA, whereas serum total PSA (TPSA) and free PSA (FPSA) were estimated by electrochemiluminescence immunoassay. A significant rise of fasting plasma glucose along with prominent dyslipidemia was observed in breast tumor cases. Marked rise in serum testosterone as well as TPSA and FPSA was documented in both benign and malignant breast tumor cases. Serum testosterone revealed a significant positive association with both TPSA and FPSA pointing towards an etiological association between them. However, surgical removal of tumor mass resulted in a marked decline of presurgical value of both TPSA and FPSA with a non-significant fall in serum testosterone revealing tumor tissue as the source of FPSA and TPSA. Thus, estimation of PSA provides prognostic information that may assist in future treatment.

Keywords Breast tumors · Testosterone · TPSA · FPSA

Introduction

Breast cancer continues to be a significant health threat, being the second most common cancer among Indian women [1]. Prostate Specific Antigen (PSA), a 33 kDa serine protease which has already been established as a valuable marker for screening, diagnosis and management of prostate cancer [2, 3] has been suggested to have a number of potential roles in breast cancer. PSA, found in a very low but detectable levels in the circulation of women, is likely to be originated from breast tissue [4]. Similar to prostate PSA, production of breast PSA is said to be under hormonal control and androgens in particular are believed to upregulate the expression of PSA gene through androgen receptor [5, 6].

Various authors have documented that free form is the predominant molecular form of PSA in breast cancer whereas the bound form is more prevalent in benign breast diseases and healthy females [7, 8]; while many others have failed to establish any such observations regarding the various molecular forms of PSA in breast cancer and benign breast diseases [9, 10]. Moreover, a fall in serum PSA levels after surgical removal of tumor tissue has been observed in some studies, while others have failed to observe any such change in serum level of PSA after surgery [8, 11].

The above observations developed an interest to conduct a study to estimate serum free PSA and total PSA along with serum testosterone in benign breast disease and breast cancer cases and to find out their association with the disease process.

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Materials and Methods

The study was conducted in the Department of Biochemistry, S.C.B. Medical College, Cuttack during the period May 2006–August 2007 in collaboration with Acharya Harihar Regional Cancer Centre, Cuttack.

Women in the age group of 20–60 years with complains of tumorous growth in breast attending the OPD of Acharya Harihar Regional Cancer Center, Cuttack were subjected to histopathological analysis and were classified into benign and malignant growth according to WHO classification [12]. Our study enrolled 34 malignant breast disease cases and 26 benign breast disease cases along with 33 age and sex matched healthy controls for evaluation and comparison. Clinical staging of the malignant cases was done according to American Joint Committee on Cancer Staging [13].

Both control and cases were subjected to estimation of parameters such as fasting plasma glucose, lipid profile, serum urea, serum creatinine along with estimation of serum total prostate specific antigen (TPSA), serum free prostate specific antigen (FPSA) and serum testosterone prior to initiation of treatment. 14 out of 34 malignant breast tumor cases and 10 out of 26 benign breast tumor patients were again analyzed for serum Testosterone, serum TPSA and serum FPSA 3 months after surgical removal of tumor tissue.

FPG, lipid profile, serum urea and creatinine were estimated in Flexor XL autoanalyzer. Serum Testosterone was estimated by ELISA [14]. Determination of serum Total PSA and Free PSA was performed by electrochemiluminescence immunoassay method [15].

Women using OC pills; hormone replacement therapy; steroid medications; with a past/present history of any other gynecological/other malignancies and presence of any chronic diseases like diabetes, renal, hepatic or thyroid disorders were excluded from the study. All those cases where the value of the said parameters was below the minimum detection limit of the assay procedure (1 ng/l for total PSA and 10 ng/l for free PSA) were adjusted to zero.

The results obtained in different groups were compared and statistically analyzed by SPSS 11 using Scheffe's test and Mann–Whitney U Test for parametric and non-parametric data, respectively. Correlation study between serum total and free PSA with serum testosterone was done by Spearmann correlation study as they were non-parametric analysis. Comparison between before surgery and after surgery values of these non-parametric data were done by Wilcoxon signed rank test for two sample comparison of paired cases.

The study has been approved by institutional ethical committee.

Results

The present study revealed (Table 1) a marked rise in FPG ($P < 0.001$) as well as serum total cholesterol and TG in cases in comparison to controls. Serum HDL and serum LDL also registered significant differences in both malignant and benign cases in relation to control group whereas noticeable rise was observed in serum VLDL only in malignant cases. Malignant breast disease cases documented a prominent rise in serum urea with no significant alteration in serum creatinine as compared with control.

Median value of serum testosterone was 0.5 ng/ml in controls and 1.3 and 2.0 ng/ml in benign and malignant cases, respectively, revealing a prominent rise in both benign and malignant groups in comparison to control ($P < 0.05$). Serum testosterone level when analyzed in the study population in relation to menopausal status, the present study registered a high serum testosterone value in premenopausal benign breast disease cases than postmenopausal group. In malignant breast disease cases, serum testosterone was found to be higher in postmenopausal group in comparison to premenopausal cases. (Chart 1).

A prominent rise in serum Total PSA was observed in both benign and malignant breast disease cases in comparison to controls. Similarly serum Free PSA registered a marked rise in cases when compared with controls. However, no significant difference was documented in both serum TPSA, FPSA levels between benign and malignant breast disease cases (Table 2).

No statistically significant difference was observed in both serum TPSA and FPSA in the study population, taking into consideration their menstrual status, exhibiting no association of these parameters with menstrual status by

Table 1 Serum biochemical parameters in the study population

Parameters (mg%)	Controls (n = 33)	Benign (n = 26)	Malignant (n = 34)
FPG	72.3 ± 8.6	88.4 ± 10.8*	88.64 ± 8.4*
Chol.	169.8 ± 16	184.0 ± 10.7*	200.8 ± 7.7*
TG	111.2 ± 16.1	117 ± 15.6	169.4 ± 35.9*
HDL	46.1 ± 6.5	40.5 ± 3.9*	32.14 ± 4.1*
LDL	101.2 ± 14.9	119.6 ± 10.6*	135 ± 7.5*
VLDL	22.4 ± 3.35	23.37 ± 3.27	33.9 ± 3.5*
Urea	24.7 ± 4.4	26.7 ± 5.0	35.7 ± 5.2*
Creatinine	1.1 ± 1.4	0.96 ± 0.23	1.14 ± 0.37

* $P < 0.001$

Chart 1 Serum testosterone in the study population according to menopausal status

Table 2 (a) Serum total PSA and free PSA in the study population

Groups	Total PSA (ng/l)		Free PSA (ng/l)	
	Median	Range	Median	Range
Controls (<i>n</i> = 33)	0	0–50	0	0–25
Benign (<i>n</i> = 26)	12	0–72	0	0–35
Malignant (<i>n</i> = 34)	15.5	0–119	0	0–80

(b) Statistically significant difference between the groups

	Total PSA	Free PSA
Controls vs benign	$P < 0.01$	$P < 0.05$
Controls vs malignant	$P < 0.01$	$P < 0.05$
Benign vs malignant	ns	ns

Analysis done by Mann–Whitney U test

Mann–Whitney U test (Table 3). Spearman correlation analysis revealed a significant positive association between serum Testosterone and TPSA (Graph 1, $r = 0.399$, $P \leq 0.01$). A similar positive association was also documented between serum Testosterone and FPSA (0.294, $P < 0.01$) in the study population pointing towards an etiological association between them.

When both benign and malignant breast disease cases were treated with surgery of tumour mass, pre and post surgery analysis of both serum TPSA and FPSA registered a significant decline of their pre-surgical value after surgical removal of tumour mass by Wilcoxon signed rank test (Table 4) whereas no prominent fall was observed in serum Testosterone level after surgery in both benign and malignant cases.

Table 3 Serum total PSA and free PSA in the study population according to menstrual status

Groups	Total PSA (ng/l)		Free PSA (ng/l)	
	Median	Range	Median	Range
Controls (<i>n</i> = 33)				
1. Premenopausal (<i>n</i> = 19)	0	0–50	0	0–20
2. Postmenopausal (<i>n</i> = 14)	2.5	0–40	0	0–25
	ns		ns	
Benign (<i>n</i> = 26)				
1. Premenopausal (<i>n</i> = 19)	15	0–72	0	0–35
2. Postmenopausal (<i>n</i> = 7)	8	0–52	0	0–20
	ns		ns	
Malignant (<i>n</i> = 34)				
1. Premenopausal (<i>n</i> = 18)	19	0–91	0	0–80
2. Postmenopausal (<i>n</i> = 16)	9.5	0–119	0	0–80
	ns		ns

Graph 1 Correlation between serum total PSA and serum testosterone in the study population. Spearman' rank correlation coeff. = 0.399

Table 4 Serum total PSA, serum free PSA and serum testosterone in the benign and malignant breast tumor cases before and after surgery

Groups	Total PSA (ng/l)		Free PSA (ng/l)		Testosterone (ng/ml)	
	Median	Range	Median	Range	Median	Range
Benign (<i>n</i> = 10)						
Before surgery	46	(8–72)	21	(0–35)	1.6	(1.1–2)
After surgery	30*	(0–48)	0*	(0–10)	1.5	(1–2)
Malignant (<i>n</i> = 14)						
Before surgery	30	(12–119)	20	(0–80)	2.1	(0.6–2.5)
After surgery	23.5*	(0–75)	0*	(0–20)	2.0	(0.5–2.5)

* $P < 0.05$

Discussion

PSA is one of the most valuable serum tumor markers having been used successfully for diagnosis and post-surgical management of prostate cancer. Routine use of PSA immunoassays has proven the improved rate of detection of prostate cancers. Measurement of various molecular forms of PSA has now been introduced into clinical practice by various researchers [16, 17].

The present piece of work has been taken up with an objective to measure relative proportion of the molecular forms of PSA in serum along with serum testosterone in benign and malignant breast tumour cases and to analyze their association with the severity of the disease process.

The significantly higher fasting plasma glucose in breast tumor cases in the present study may be explained by the opinion of other authors that hyperinsulinemia and insulin resistance are the causative factors in breast cancer cases. The role of Insulin Like Growth Factors (IGF) and estrogen on insulin level has also been suggested for the alteration of glucose metabolism in breast cancer cases [18–20]. Several studies have also documented an association between dyslipidemia and breast cancer [21, 22]. Increased intake of calories and fat in higher socio-economic group is cited to be the cause of increased estrogen level by the phenomenon of aromatization in adipose tissue which in turn increases the risk of breast cancer [23].

A higher level of serum testosterone in postmenopausal breast cancer cases compared to premenopausal group is in agreement with others postulations [24, 25] that the discrepancy is due to slowed metabolic removal of testosterone in post menopausal breast cancer women. High circulating level of testosterone in pre and post menopausal breast cancer cases registered by various researchers [24, 26] shows etiological role of androgens in the genesis of breast cancer.

The marked rise in serum total PSA and serum free PSA in the study group with no significant difference between the benign and malignant cases is in agreement with various authors who have registered a rise in serum total PSA and free PSA in women with tumorous growths in breast in comparison to those with no breast pathology [8]. This could be attributed to disrupted hormonal balance in such cases leading to aberrant expression of hormone dependent genes like PSA which is normally under hormonal control and upregulated by androgens and progesterone [5, 27, 28]. Many other authors have, however, failed to depict any such rise in PSA level in breast tumor cases [9, 10]. However, we observed no significant difference in total PSA and free PSA level in the study population when their menstrual status was considered exhibiting no association of the parameters with the menstrual status.

A significant positive correlation of serum testosterone with both serum total PSA and serum free PSA in the present study explains the stimulatory effect of androgens on production of PSA [6, 28]. Steroid receptor cell lines like BT 474, T-47D and MCF-7 when stimulated by androgens, progestins and glucocorticoids, produced PSA in the culture medium. This provides evidence that androgens, progestins and glucocorticoids share the same HRE in DNA and that HRE is probably associated with the PSA gene and upregulates its expression [27].

In post-operative follow up of 14 breast cancer and 10 benign breast disease cases, reevaluation for serum total PSA and serum free PSA registered a significant fall in their post surgical levels compared to their pre surgical value whereas decline in serum testosterone was not so prominent. The fall in all the three parameters points towards an association between testosterone and PSA. Observations being in agreement with that of several authors [8, 11] indicate breast tissue to be the source of PSA in females as it is one of the major hormone responsive organ in the female body. The marked decrease in their blood level after surgery prove them to be a suitable marker for monitoring the response to treatment and early detection of recurrence as suggested by Hautman et al. [11].

Thus PSA, considered to be highly tissue specific have been recommended to be an ideal tumour marker for breast malignancies. Prominent rise of TPSA and FPSA in breast tumour cases as well as the significant fall in both parameters after surgical removal of tumour tissue establishes breast tissue to be the source of PSA. The significant positive association between free and total PSA with serum testosterone strengthens the regulatory role of testosterone on the production of PSA, sharing the common HRE (hormone response element) in DNA with progestins and glucocorticoids [27].

The association of PSA with the disease process of breast tumour may be explained by its versatile biological role such as:

- (i) PSA acts as mitogen to breast tissue through of TGF- β (transforming growth factor β), a known mitogen of breast tissue [29]
- (ii) PSA degrades fibronectin and laminin, the cellular matrix proteins, thereby facilitating local invasion [30]
- (iii) It increases IGF-1, a proven mitogen of breast tissue [31]
- (iv) It degrades IGFBP-3, which normally induces apoptosis in breast tissue and its degradation by PSA stimulates tumour progression [32].

However, the connection between PSA expression and the pathophysiology of breast cancer remains to be

established. Many authors have observed that PSA possesses anti-angiogenic properties and breast cancer cases having higher levels of PSA shows better prognosis [33]. Considering the heterogeneity of the population of breast cancer cases, additional evaluation of PSA in relation to stage of tumor, risk of relapse and metastasis is required to reach at a definitive conclusion regarding assessment of its utility as a diagnostic and prognostic marker in breast cancer.

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