Reconnoitring the Status of Prostate Specific Antigen and its Role in Women

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Abstract Prostate specific antigen is considered to be a tumour marker having maximum utility and specificity for prostate cancer since decades. After the discovery of methods to quantify different molecular fractions of prostate specific antigen (PSA), its usefulness in diagnosing early prostate cancer cases has increased tremendously. The “specificity” of PSA, is now challenged by many studies which proved that PSA, once believed to be secreted exclusively by prostatic epithelium, is also present in females. The exact biological role of extraprostatic PSA is still debatable though many theories substantiated by in vitro evidence has been put forward. With the advent of ultrasensitive analytical techniques, PSA is now quantifiable in female serum in its various molecular forms and this has led to many assumptions of it being useful as a marker in female breast cancers. In a similar scenario to prostate cancer, the ratio of free to total PSA is shown to be useful in detecting early breast cancer cases. It is also shown to be a good prognostic indicator and a predictor of response to therapy and recurrence. Apart from its role in breast cancer, it has been advocated to be a marker of hyper androgenic states in women like hirsutism and polycystic ovarian syndrome. Conflicting reports regarding the role of extra prostatic PSA is accumulating but it has been proven beyond doubt that PSA is no longer specific and confined to prostate gland. Various studies have registered that PSA is an ubiquitous molecule, secreted by hormone responsive organs and its synthesis is stimulated by androgens and progesterone but not oestrogens. In this article, a review of various literatures is done about the presence of extra

prostatic PSA, its probable role in those sites as well as its utility as a tumour marker in breast cancer.

Keywords Prostate specific antigen · Tumour marker · Breast cancer · Fibroadenoma · Hyperandrogenism · Specificity · Sensitivity

Tumour markers are biomolecules present in blood or tissue, associated with a particular cancer, estimation or detection of which helps in the diagnosis and management of the malignancy.

The first tumour marker used in clinical practice was Bence Jones Protein, discovered in 1847 as a diagnostic test for multiple myeloma. The knowledge about tumour markers were further expanded by the use of hormones, enzymes, isoenzymes, and proteins as tumour markers as well as commencement of chromosomal analysis of tumours, discovery of oncofetal antigens like CEA and AFP and the detection of these antigens by the use of monoclonal antibodies. Advances in techniques of molecular biology lead to the genetic analysis and use of molecular probes to study oncogenes and tumour suppressor genes. With technological progress, levels of certain genetic materials can now be measured in blood and this shifted the focus of tumour markers from the traditional proteins, hormones and enzymes to the measurement of genetic materials like DNA, RNA, microRNA and gene analysis. And while it has been hard to identify a single substance that provide conclusive information about a certain type of cancer, scientists now look at patterns of genes or proteins in the blood as potential tumour markers.

The significance of early diagnosis of a cancerous growth needs no special mention. A tumour marker with sufficient specificity and sensitivity is still far from our

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reach. The only exception was supposed to be Prostate Specific Antigen (PSA), which was said to be “specific” for prostatic diseases in males, hence the name.

History of PSA: In the 1960s Ablin et al. [1] reported about a novel protein found in seminal fluid and this finding was corroborated by Hara et al. [2] in 1971 as a protein unique to semen. They named the protein as “gamma seminoprotein”. Later, Li and Beling [3] in 1973 isolated and purified the protein. In 1978, Sensabaugh [4] characterized it as a 30 Kda semen specific protein. In 1979 Wang et al. [5] using gel electrophoresis technique isolated the tissue specific antigen and named it Prostate Specific Antigen (PSA) owing to its specificity of origin in prostate gland of males. Further studies by them documented that PSA was immunologically identical to the protein discovered by Hara and Sensabaugh. In 1980, Papsidero and Wang et al. [6] measured the level of PSA in serum by developing a serological test. The first definitive clinical study investigating the utility of PSA in prostate cancer was done by Stamey et al. [7] at Stanford University. Since then PSA has gained tremendous importance in the arena of prostate cancer diagnosis and prognosis, with its various molecular forms now abetting in the differentiation of prostate cancer from benign prostate diseases.

Biochemistry of PSA: PSA, a 33 kDa glycoprotein containing 237 amino acids, 4 carbohydrate side chains and multiple disulphide bonds is homologous with the proteases of the kallikrein family and hence called human glandular kallikrein-3 (hk-3). It is a serine protease which splits the seminal vesicle proteins seminogelin I and II resulting in liquefaction of the seminal coagulum. The gene encoding PSA has been sequenced and localized to chromosome 19.

Molecular forms of PSA in the serum: Larger fraction is in bound form complexed to either alpha 2 macroglobulin (AMG) or alpha 1 anti-chymotrypsin (ACT) and a smaller fraction is present in the free form. Out of the two bound forms, PSA–ACT can be measured by immunoassays as it has two unmasked epitopes whereas PSA–AMG has no epitopes exposed for detection. The bound form of PSA with ACT or AMG leads to its diminution of activity but it is not clear as to what extent the free PSA possess enzymatic activity. Various studies have documented that the free PSA represents the zymogen [8] or nicked [9] form of PSA with no enzymatic activity and it doesn't form any complexes with the protease inhibitors. Stamey et al. [7] found the half-life of free PSA to be 2.2 ± 0.8 days and cleared by renal clearance while Oesterling et al. [10] determined the half-life of total PSA (PSA–ACT + free PSA) to be 3.2 ± 0.1 days. The bound form being of higher molecular weight is not filtered by kidneys but undergoes hepatic clearance by a receptor mediated

endocytosis or through macrophage activity of Kupffer cells [11].

Role of PSA in body: PSA is one of the major proteins of seminal fluid and expressed by the epithelial cells lining the acini and ducts of prostate gland. It liquefies the seminal coagulum by degradation of fibronectin and seminogelin I and II leading to release of free motile spermatozoa [12]. Iwamura et al. [13] further demonstrated that the parathyroid hormone related protein (PTHrP) is a seminal plasma protein which shows strong amino acid similarity with Parathyroid hormone and participated in the regulation of calcium level in seminal fluid. PTHrP is also a substrate of PSA and cleaved by it though an exact ramification of this action of PSA is not yet clear. PSA enzymatically digests IGFBP-3, thus decreasing their serum level and increasing the bioavailability of IGFs [14]. It has also been seen to activate urokinase type plasminogen activator uPA, the enzyme responsible for conversion of plasminogen to plasmin [15]. PSA has been reported to hydrolyse Insulin A and B chain, gelatin, recombinant interleukin 2, myoglobin, ovalbumin and fibrinogen [16]. Webber et al. [17] suggested that extracellular matrix protein laminin is also a proteolytic substrate of PSA. PSA also cleaves and activates TGF- β and secretory leucocyte protease inhibitor (SLPI) although the physiological significance of these associations is still debatable. In addition to the proteolytic role of PSA, a novel property of PSA was documented by Fortier et al. [18] in their study who evaluated the anti-angiogenic properties of PSA. Based on the observation that patients with breast cancer having higher levels of PSA, had a better prognosis, they hypothesized that PSA may have anti-angiogenic properties. To test this concept, they evaluated the effects of PSA on endothelial cell proliferation, migration and invasion by treating bovine and human endothelial cells with purified human PSA and then stimulated them with FGF-2 and VEGF. They found that PSA inhibited endothelial cell proliferation to both FGF-2 and VEGF stimulus. In yet another experiment to evaluate the ability of PSA to inhibit lung metastasis of melanoma cells, the investigators administered BI6BL6 melanoma cells intravenously to mice and then gave PSA for 11 consecutive days and found a 40 % reduction in the mean number of lung tumour nodules compared to saline treated controls. Later they established in vivo that PSA inhibited FGF mediated angiogenesis in a Matrigel plug assay by using a murine model [19]. The authors concluded that, in addition to its other physiological functions, PSA may act as an endogenous anti-angiogenic protein. Similarly, Mattsson et al. [20] in their studies suggested that PSA exerts anti-angiogenic activity related to its enzymatic activity and hence it might be associated with the slow growth of prostate cancer.

PSA in Prostate Cancer: The introduction of PSA in clinical practice has greatly increased the detection of localised prostate cancer. Normal serum level of PSA in males is <4 ng/ml. Nearly one half of all the cancers detected because of an elevated PSA level are localized and these patients are candidates for potentially curative therapy. A major limitation of PSA as a prostate cancer marker is the overlap in values between benign hypertrophy of prostate and prostate cancer when the level is between 4 and 10 ng/ml. With the introduction of the various molecular forms of PSA and their assay made possible, differentiating BPH and prostate cancer on the basis of predominant molecular form of PSA in serum has been more accurate and specific. Stenman et al. [21] reported that men with prostate cancer had more bound PSA (PSA-ACT) than free PSA in contrast to men with BPH. While the total PSA level alone is neither sensitive nor specific enough for early diagnosis of prostate cancer, the ratio of free to total PSA improves both sensitivity and specificity with total PSA level between 4 and 10 ng/ml [22, 23].

Extra-prostatic PSA: The organ “specificity” of PSA has recently been challenged and PSA is no longer said to be confined to males and prostate gland. With the availability of highly sensitive immunoassays it has become apparent that PSA is expressed in non-prostatic tissues and most noteworthy is that it is now quantifiable in females. Several investigators have reported the presence of PSA in other tissues and biological fluids including breast cyst fluid, amniotic fluid, breast milk, nipple aspirate fluid, pituitary, endometrium, ascetic fluid and CSF [24–26]. PSA expression has also been reported in a wide variety of tumours by immunoassay as well as RT–PCR methods. Bodey et al. [27] detected PSA immunocytochemically in primary and metastatic melanomas. Strengthening the fact that PSA is an ubiquitous molecule and not restricted to prostate gland alone was the observations of Zarghami et al. [28] who detected PSA in lung tumours and normal lung tissue. Similarly, Clements et al. [29] in their study registered the presence of PSA mRNA in some pituitary tumours by RT-PCR. Organ specificity of PSA was again challenged by the observation of Yamamoto et al. [30] in their published case report of a patient suffering from adenocarcinoma of colon who had a very high level of serum PSA and a significant decrease in the serum level after surgery indicating the tumour to be the source of PSA in the patient.

PSA expression in male breast cancer: Male breast cancer is uncommon, accounting for less than 1 % of all breast cancers. Metastatic prostatic cancer seeded to breast and primary breast cancer may be histologically indistinguishable without immunohistochemistry but

differentiating between primary and metastatic disease within the breast is important as the treatment options for each are radically different. Carder et al. [31] reported a case of prostatic carcinoma metastasising to the breast, which was at first diagnosed to be a primary breast cancer but subsequently, was confirmed to be a metastatic case from prostate cancer only after performing immunohistochemistry for PSA and PSAP, after a review at multidisciplinary meeting. After the recent knowledge about PSA expression in female breast cancers they investigated a small series of male breast cancers for PSA and PSAP expression. They found focal PSA expression in one of 11 cases examined; endorsing that PSA may be expressed in male, in addition to female breast cancer. A few other studies too found PSA expression in male breast carcinoma [32–34]. However, many of the studies found that expression of prostatic acid phosphatase (PSAP), another marker of prostate cancer was entirely absent in all the primary male breast cancer cases who were positive for PSA, whereas PSAP was strongly positive in case of metastatic prostate cancer seeded to breast. Therefore, they concluded that there are limitations of the marker PSA in differentiating primary breast cancer from metastatic disease in men which must be recognised and emphasised in the context of small potentially unrepresentative core biopsies (Table 1).

PSA in females: Diamandis et al. [35] first revealed that low levels of PSA are released into the female serum, the level of which is about 1000-fold less than those in male serum. Nipple aspirate fluid has the second highest PSA concentration after seminal plasma while the third highest level is found in breast milk of lactating women [36]. Females lack prostate gland hence the exact source of PSA in females is still contentious. Wernert et al. [37] proposed periurethral glands to be the female prostate as 67 % of them stained positive for PSA with a histological appearance similar to that of the prostate gland while Clements and Mukhtar [38] reported the presence of PSA in normal endometrium tissue and suggested it to be a regulator of uterine function. Researchers demonstrated that PSA level varies with the phase of menstrual cycle observing a rise in serum PSA level with the progesterone peak with a 10–12 day lag period, suggesting that PSA in females is regulated by corpus luteum steroids [39]. It was also reported that women with elevated androgen levels, consequently exhibiting hirsutism also had elevated levels of PSA with the bound form PSA-ACT as the major molecular form present in them [40]. Mannello et al. [41] reported a case of female breast intracystic carcinoma with high amounts of PSA in the aspirated cystic fluid. Tumour extract analysis revealed the presence of both estrogen and

Table 1 Extraprostatic PSA

Serial no.	Authors	Study groups	Source/tissue
1.	Melegos et al. [25]	Normal and abnormal pregnancies	Amniotic fluid
2.	Bodey et al. [27]	Human primary and metastatic melanomas	Biopsy specimen for immunocytological detection of PSA
3.	Zarghami et al. [28]	Lung tumours	PSA mRNA in tumour tissue
4.	Clements et al. [29]	Human pituitary tissues	Kallikrein gene expression in tumour tissue
5.	Yamamoto et al. [30]	Adenocarcinoma of the colon	Serum PSA
6.	Manello et al. [24]	Ascitic fluid	Ascitic fluid PSA
7.	Clements et al. [38]	Healthy females	Endometrial tissue
8.	Bayatti et al. [57]	Polycystic ovary syndrome and hirsutism	Serum PSA
9.	Obiezu et al. [59]	PCOS patients	Urine PSA
10.	Borchert et al. [43]	Fibroadenomas and breast cysts	Serum PSA
11.	Wernert et al. [37]	Healthy females	Periurethral glands
12.	Carder et al. [31]	Male breast cancer	Immunohistochemistry for PSA
13.	Diamandis et al. [45]	Fibrocystic breast diseases	Breast cyst fluid
14.	Zarghami et al. [39]	Healthy females	Serum PSA

progesterone receptors and high quantities of PSA which was predominantly in the free form. Their data support the contention that PSA immunoreactivity in intracystic fluid of breast carcinoma is partly the result of secretory activity by the neoplastic cells and that the steroid receptors modulates its expression.

It has been reported that the normal level of PSA in women is $\leq 0.01 \mu\text{g/L}$ [42]. It was observed that around 7 % women with benign breast disease had a serum PSA level of $>30 \text{ ng/L}$ in comparison to 1.5 % of normal women detected with that level [36, 43]. The studies also documented that the predominant molecular form of PSA in women with benign breast disease was free PSA and concluded that circulating level of serum PSA in women with fibroadenoma or breast cysts was very high reaching to a value comparable with male sera. Radowski et al. [44] opined that PSA may be used as a marker for benign breast disease like breast cyst and fibroadenoma as the level of PSA in these patients are considerably higher than women with no breast pathology. Diamandis et al. [45] demonstrated large amounts of PSA up to $82 \mu\text{g/L}$ accumulation in breast cyst fluid and as the cyst fluid proteins are believed to be secretory products of epithelial cells surrounding the cyst, hence PSA too is assumed to be the products of breast epithelium. This finding was also substantiated by Yu et al. [46] who showed that high level of PSA is present in the cytosolic extracts of breast tissue of women with breast pathology. Since the revelation about PSA having non-prostatic source and its detection in women particularly in patients with breast pathology, its presence in breast cancers was studied and analysed by many scientists and was of concern to the oncologists. Gau et al. [47] demonstrated that expression of PSA in prostate

gland is under steroid regulation and can be stimulated by testosterone. This raised the assumption that whether the two tissues, prostate and breast share a steroid stimulated regulation of PSA expression as the molecular weight and mRNA sequence of breast PSA were found to be identical to seminal PSA [48, 49]. Ferguson et al. [50] reported that PSA immunoreactivity is significantly associated with progesterone and oestrogen receptor positivity of tumour cells in breast cancer. It was subsequently proved beyond doubt by many studies that production of PSA in breast tumours are under the control of androgen and progesterone receptors [51, 52]. When the steroid hormone receptor—positive cell lines T-47D and MCF-7 were treated with androgens, progestin or glucocorticoids, stimulation and production of PSA in the culture medium [53] was observed. In the T-47D this stimulation was found to be blocked by oestrogen. It is an established fact that HRE (Hormone Response Element) of androgens, progestin and glucocorticoid receptor is same and ostensibly it is this common HRE which is associated with the PSA gene. It is important to mention here that HRE of oestrogen receptor is different from the above mentioned common HRE of androgens, progestin and glucocorticoids. Some studies confirmed that extra prostatic PSA is under steroid hormonal control and androgen stimulation promotes PSA expression in women in vivo. A recent study documented that the concentration of PSA in cord blood is higher in case of higher gestational age, male baby and operative delivery and explained that higher progesterone levels in prenatal maternal blood having male babies may be responsible for the higher cord blood PSA while operative interventions like caesarian section or forceps delivery results in much more stress and strain compared to normal

vaginal delivery, with subsequent increase in levels of adrenal glucocorticoids, and therefore, higher cord blood PSA [54]. Breul et al. [55] reported that female patients receiving Testosterone supplementation has PSA levels comparable with that of men with significantly elevated urinary PSA levels presumably as a result of increased production of PSA by the periurethral glands. These studies reveal that PSA production is upregulated by androgens. A study on tissue culture system using female breast cancer cell lines demonstrated that the production of PSA in these cell lines is mediated through the action of Progesterone, androgens, mineralocorticoid and glucocorticoid receptors but not estrogen receptor. These data re-established the mechanism of gene regulation by steroid hormone receptors as PR (Progesterone receptor), AR (Androgen receptor), MR (Mineralocorticoid receptor) and GR (Glucocorticoid receptor) bind to same hormone response element HRE in DNA, which is different from the HRE of ER (Estrogen receptor). His results were buoyed by the observations made by Obeizu and his associates [56] in their study on 32 female to male transsexuals where they demonstrated an increase in both serum and urinary PSA levels of PSA post testosterone treatment and the source of this PSA was presumed to be from periurethral glands under androgenic stimulation. Melegos et al. [40] registered a significantly higher level of PSA in serum of hirsute, hyper androgenic women which correlated well with the serum level of 3α -androstenediol glucuronide which is specific metabolite of androgen action. Bayatti and his co-workers [57] evaluated total PSA in 35 females with PCOS (Polycystic Ovarian Syndrome) and hirsutism and tried to correlate it with serum testosterone. They observed that serum PSA was higher in PCOS patients with hirsutism and the level increased linearly with the score of hirsutism. They however failed to show a significant correlation between testosterone and PSA in those patients. The diagnostic value of PSA in hirsute women was analysed by Gullu et al. [58]. They reported that both total and free PSA level were found to be raised in women with hirsutism compared to controls and the PSA level was positively correlated with Testosterone level strengthening the possibility that androgens have the stimulatory effect on PSA production. From all these observations, it could be postulated that PSA can be a new marker of hyper androgenic states in women like hirsutism and PCOS [40, 57–59]. These findings lead to the speculation that any tissue that contain steroid hormone receptors has the ability to produce PSA and that PSA is an ubiquitous enzyme produced not only by breast cancer and endometrium but by many other tissues including normal breast though in much lower amount compared to that produced by prostatic cells.

In a large study by Borchert et al. [36], it was found that bound form of PSA (PSA-ACT) is the major molecular

form of PSA in normal and hirsute women whereas free form is the predominant form in benign and malignant breast diseases. The authors suggested that this differential expression of molecular forms of PSA in women with breast pathology and those with no breast pathology could be the basis of a serological test for breast cancer. Supporting the above findings, Black et al. [60] revealed in their study which included 118 breast cancer patients and 46 patients with breast cyst that free PSA is the predominant molecular form in breast cancer patients. They did a follow-up assessment of PSA level 6 months after tumour removal surgery in 115 patients out of total 118 patients enrolled and documented a statistically significant decrease of free PSA level after surgery. Similar findings were observed by Hautmann et al. [61] in their study registered a high level of PSA in breast cancer patients compared to benign breast disease cases and controls and that level decreased after tumour removal but the level didn't reach statistically significant value. Abdulnabi [62] in his study documented an increased serum free PSA level in breast cancer patients compared to patients with benign tumours of breast. They also registered a significant difference in the pre and post-surgery level of free PSA in their study on 30 breast cancer patients suggesting that free PSA can be used as both a diagnostic as well as prognostic marker in breast cancer. Mashkoor et al. [63] also documented a significant fall in the level of TPSA and FPSA after surgical removal of tumour mass in their study on 55 breast cancer patients and 82 controls. They registered a high TPSA and FPSA level in breast cancer patients which was positively correlated with younger age group and early stage of cancer. They also found FPSA to be the predominant molecular form of PSA in breast cancer patients. In a study conducted by this author and her co-workers [64], it was also found that PSA was predominantly present in free form in breast cancer patients compared to fibroadenoma cases and healthy women with no breast pathology and that free form was > 50 % of total PSA level. We also observed that the PSA level, in particular the free PSA level decreased significantly after tumour removal in breast cancer patients. On analysis we found that PSA in the free form has prodigious specificity but low sensitivity to be used as a tumour marker for routine clinical use. We attributed this to the available assay methods for free PSA (we did it by electro chemiluminescence having a detection limit of 1 ng/ml for total PSA and 10 ng/ml for free PSA) by which many cases reported a non-detectable level of free PSA as PSA level in women even with breast pathology is very low compared to male sera. We reached to a conclusion that with increased sensitivity of assay procedure, free PSA can have increased sensitivity to be used a tumour marker. Chang et al. [65] adopted a localized surface plasmon coupled fluorescence fiber-optic

Table 2 Detection of PSA in breast cancer

Serial no.	Authors	Study groups	Tissue/serum
1.	Borchert et al. [36]	Benign and malignant breast diseases, Idiopathic hirsutism, Blood donors	Serum TPSA/FPsA
2.	Zarghami et al. [51]	Breast cancer	Breast carcinoma cell line T-47D for steroid hormone regulation of PSA gene
3.	Yu et al. [46]	Breast cancer, benign breast disease and normal breast tissue	PSA in breast tissue
4.	Diamandis et al. [49]	Breast tumours	PSA immunoreactivity in breast tumour tissue
5.	Hautmann et al. [61]	Benign breast disease, breast cancer	Serum PSA
6.	Black et al. [60]	Breast cancer, breast cysts, uterine fibroids	Serum TPSA/FPsA
7.	Chang et al. [65]	Breast cancer	Serum PSA
8.	Mashkoo et al. [63]	Breast cancer	Serum TPSA/FPsA
9.	Hall et al. [52]	Androgen receptor +ve breast tumours	Breast tissue
10.	Abdulnabi [62]	Breast tumours	FPsA in breast tissue
11.	Lehrer et al. [67]	Breast cancer	RT-PCR for PSA in breast cancer tissue
12.	Bharaj et al. [77]	Breast cancer	TA repeat polymorphism of the 5alpha-reductase gene in tumour tissue
13.	Miller et al. [32]	Breast cancer	Immunohistological detection of PSA in tumour tissue
14.	Kraus et al. [34]	Male and female breast cancer	PSA and hormone receptor expression in breast tumour tissue
15.	Zhao et al. [69]	Breast cancer	Nipple fluid for CEA and PSA
16.	Dash et al. [64]	Breast cancer and Fibroadenoma	Serum total PSA and free PSA

biosensor, which combines a sandwich immunoassay with the localized surface plasmon technique, to improve the sensitivity of conventional immunoassay technology for the detection of PSA in female sera. They observed a higher PSA level in the sera of breast cancer patients compared to healthy controls (Table 2).

PSA is considered to be an IGFBP protease and researches show that the insulin like growth factors (IGFs) and their associated binding proteins (IGFBPs) are involved in breast cancer cell proliferation. Kaulsay et al. [66] investigated the level of PSA, IGFs, IGFBP 1,3 and 6 in diagnosed breast cancer patients and patients with some benign breast disease and observed that PSA level was significantly higher and IGFBPs are significantly lower in cancer patients compared to the other group and hence they opined that this finding may be having clinical implications in the disease progression in breast cancer patients as low IGFBPs level may lead to high IGF level due to increased bioavailability though in their study the difference of IGF levels in both the groups were not statistically significant. In a very novel approach Lehrer et al. [67] used RT-PCR assay to identify and amplify PSA in venous blood specimen of 78 breast cancer patients with an objective to identify the risk of recurrence in women with node negative and small tumour size breast cancer. They amplified PSA fragments from venous blood samples in 18 out of 78 patients but failed to do so from any of normal men and

women. They concluded that PSA RT-PCR may be useful for determining the presence of circulating metastatic cells in some women with node negative breast cancer and therefore the potential for these women to develop recurrence in them is higher and hence should be meticulously followed up and given adjuvant therapy.

In a study by Foekens et al. [68] the authors observed that the level of PSA in cytosols of primary breast tumors might be a marker to select breast cancer patients who may benefit from systemic Tamoxifen therapy envisaging its role as an independent variable of poor prognosis.

The dispute on the role of PSA in extra prostatic tissues and its benefit as a tumour marker in breast cancer continues. While some studies found it to be promising candidate in the arena of tumour markers in breast cancer detection, several other studies annulled its role as a tumour marker of clinical importance in breast cancer. In a study on PSA level in nipple aspirate fluid from women with breast cancer, breast proliferative lesions (ductal and lobular carcinoma in situ and atypical ductal hyperplasia) and with no known breast pathology, Zhao et al. [69] revealed that (Nipple Aspirate Fluid) NAF PSA titres did not seem to be beneficial for breast cancer detection. Narita et al. [70] made a comparative study on PSA with other established markers of breast cancer like ER/PR status, Her2/neu status, tumour size, nodal and menopausal states and reached to a conclusion that PSA doesn't have any

advantage over the established prognostic markers of breast cancer and hence cannot be used as an independent prognostic factor in breast cancer. Serum PSA levels in breast cancer were examined with high sensitivity chemiluminescence assay to determine their efficacy as a tumour marker in breast by Romppanen et al. [71] in 90 histologically proven breast cancer patients, 94 women with benign breast diseases, and 100 controls. They observed that PSA exhibited no relation with the histological type or grade or stage of breast cancer but recorded a low PSA level in healthy women after menopause which failed to decrease in patients with breast cancer or benign breast disease. In their conclusion, they said that PSA have no utility in distinguishing healthy women from women having benign or malignant breast diseases. In our study [64] we didn't register any difference in PSA level between pre and post-menopausal patients with breast cancer believing that menstrual status has no role in the secretion of PSA in females with breast pathology and similar observations were earlier made by Giai et al. [72] who also didn't not find any difference in the PSA level in normal healthy women and breast cancer patients in the post-menopausal group. Though they concluded in their study that the major circulating form of PSA in breast cancer patients is free PSA (mol.wt. approx. 33,000 Kda), still they could not find any association of tumour PSA level and serum PSA level in those patients. They also didn't register any difference of PSA concentration in pre and post-surgical sera of breast cancer patients. Hence they opined that PSA lacks sufficient potential to be used as a tumour marker for breast cancer diagnosis or monitoring. They however viewed tumour PSA concentration as a favourable prognostic indicator in women with breast cancer. Breast cancer associated with high levels of PSA have been shown to be an indicator of favourable prognosis. The relative proportions of the molecular forms of PSA, already been established as marker for clinical differentiation between prostate cancer and benign prostate hypertrophy also displays potential in differentiating between women with and without breast cancer. Many inputs from various studies show that the difference in the relative proportions of the molecular forms of PSA in females may have potential diagnostic applicability in breast cancer.

Suggested Role of PSA in the Pathogenesis of Breast Cancer

The exact role of PSA in breast tissue is not yet clear and data regarding the enzymatic activity of mammary PSA is not substantial but a number of studies documented some potential role of PSA in breast cancer pathogenesis like:

1. PSA induces irrepressible proliferation of osteoblast and fibroblast cell lines [73]
2. PSA degrades extracellular matrix glycoproteins like fibronectin and laminin and hence is a potential modulator of matrix degradation leading to epithelial basement membrane destruction and facilitating local invasion. PSA also stimulates cell detachment which is evocative of its role in tumour progression and metastasis [17].
3. PSA is an IGFBP-3 protease, and the proteolysis of IGFBP3 (Insulin like growth factor binding protein-3) by PSA consequently decreases the affinity of this binding protein for IGF-1 (Insulin like growth factor-1). This leads to a concomitant elevation of free IGF level in serum which is a known mitogen of breast cancer cells [74].
4. In addition to the aforesaid role of IGFBP-3 as a binding protein of IGF, different studies suggest that IGFBP-3 may induce apoptosis in breast cancer cell lines and thus has antiproliferative effects on breast cancer cells [75, 76]
5. Many studies also documented a unique and novel role of PSA having antiangiogenic property highlighting the fact that tumours expressing more PSA has a better prognosis and slow growth compared to those having lower levels and they attributed this to the antiangiogenic property of PSA as angiogenesis is essential for rapid tumour progression [19, 20]. A positive association between higher concentration of PSA and better prognosis of breast cancer was also registered by Bharaj et al. [77] in their study. 5α reductase catalyzes the reduction of testosterone to its more bioactive form of dihydrotestosterone in many androgen dependent tissue. The bioactive form of testosterone then activates many genes including that of PSA. The 3'UTR of the 5α reductase gene contains either no TA (TA_0) repeats or it has 9TA (TA_9) repeats or 18TA (TA_{18}) repeats and that variations in these dinucleotide repeats influences the activity of 5α reductase. The researchers observed a statistically significant association between high PSA concentration and a heterozygote (TA_0)/(TA_9) or a homozygote (TA_9)/(TA_9) along with a reduced risk of relapse in the DNA of less advanced breast cancer patients compared stage III or IV patients. There was no (TA_{18}) alleles detected in either breast cancer cases or healthy controls.

PSA expression in females is stimulated by androgens and progestin. Breast cancer associated with high levels of PSA have been shown to be an indicator of favourable prognosis. The relative proportions of the molecular forms of PSA, already been established as marker for clinical differentiation between prostate cancer and benign prostate

hypertrophy also displays potential in differentiating between women with and without breast cancer. Many inputs from various studies shows that the difference in the relative proportions of the molecular forms of PSA in females may have potential diagnostic applicability in breast cancer. An ideal tumour marker with sufficient diagnostic sensitivity and specificity is of immense value for early diagnosis of breast malignancies in order to reduce the morbidity and mortality associated with breast cancer. Prostate specific antigen (PSA), particularly the free form do exhibit potential to be used as a diagnostic and prognostic indicator in breast cancer but its clinical utility is limited at present as the results and inputs of various studies in this regard has been still insufficient and inconclusive.

Further studies with larger cohorts of patients and longer period of follow up along with high sensitivity detection assay methods and combination with other already known tumour markers can establish the clinical utility of PSA in breast cancer and afford unassailable evidence for its diagnostic and prognostic use as a weapon against early detection of breast cancer occurrence and recurrence thereby substantially decreasing the rate of morbidity and mortality associated with this dreadful disease.

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