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Prostate specific antigen: a new means as diagnostic and prognostic factor for breast cancer

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ABSTRACT

Prostate specific antigen (PSA) is a serine protease expressed at high levels in prostate epithelium and elevated PSA in serum is a well established marker, and also helps in management of prostate cancer. In recent times it has become widely accepted that PSA is also present in many non prostatic sources, creating doubts about the specificity of its tissue expression. Numerous studies have suggested that the molecular forms of PSA imply to signify a potential tool for the risk assessment of breast cancer. Studies have suggested new biological role of PSA in breast physiopathology. Additional studies are essential to enrol PSA indisputably as an additional means as diagnostic and prognostic tool for breast cancer. Here is the summary of how PSA has a potential to become a new diagnostic and prognostic tool.

Keywords: Breast cancer, Prostate Specific Antigen, Free PSA, Total PSA.

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INTRODUCTION

The leading and well established screening techniques for breast cancer are mammography and physical examination, which cannot identify early breast cancer and may miss 10-40% [1]. Other type of identification of breast cancer is use of markers like carcinoembryonic antigen, carbohydrate antigen 15.3, tissue polypeptide specific antigen and mammary serum antigen. Anyhow the sensitivity of these markers is limited.(?) Therefore there is need to develop a new means to diagnose breast cancer. The new means of today's scientific world may be prostate specific antigen [PSA]. From the studies carried out by different researchers we can say that PSA has the potential as diagnostic and prognostic tool for breast cancer.

PSA was 1st discovered in 1970's; [4]. PSA is a serine protease as the name says it was thought to be produced or expressed and specific for prostate gland. studies carried out by many researchers in the past 2 decades has showed that this serine protease is not unique to prostate gland itself but it is multifunctional and produced in female tissues like breast tissue. [5]. PSA in female tissue was 1st seen to produce in peri urethral [skenes gland], [7]. Referred as female prostate because of its similar origin and development to that of male prostate[8]. It is said that PSA is produced in female tissues by hormonally regulated tissues like breast [9]. PSA is seen in breast tumours, [9-12] breast cysts,[13,14] hyper plastic breast tissue [9] breast secretions like milk of lactating women,[15] nipple aspirate fluid. [16-18].

PSA is also known as human glandular kallikrein 3 [hK3], gene locus is located on chromosome 19q13.3to q13.4, which has a region of 261558bp [20].

In vitro studies have confirmed PSA gene expression in breast cancer cell lines which are steroid hormone receptor positive is under hormonal control and can be induced by androgens , progestin's , mineral corticoids and glucocorticoids [6]. From the studies of E.P. Diamondis et al., it is clear that PSA exist in different molecular forms namely, Free PSA and Bound PSA. Total PSA=Free PSA+ bound PSA.

Bound PSA is nothing but PSA bound to proteinase inhibitors $\alpha 1$ – antichymotrypsin [PSA-ACT].

Molecular weight of Free PSA is 33KDa and PSA-ACT is 100KDa [21]. Here we are discussing about PSA produced by female tissues and non prostatic PSA and its use as new diagnostic and prognostic means for breast cancer.

Identification of PSA in Female Breast Tissue and Serum

The idea of specificity of kallikrein for prostate gland was changed when PSA expression was 1st discovered in apocrine foci of female fibrocystic breast tissue in1989, using immunohistochemical reactions. Presence of PSA was indentified in more than 30%of breast cancer cytosolic extracts by Diamandis et al., in 1994. Different analysis like Western blotting,

chromatography have shown that molecular mass of breast tumour PSA and prostatic PSA were identical [24]. Serum PSA concentration levels in breast cancer patients were also indentified using HPLC and immunoflurometric assay. Studies have shown that in female breast tumours and serum of female breast cancer patients, Free PSA is the major fraction when compared to male serum in which PSA-ACT is found to be predominant [22].

PSA In Breast Secretions

Few studies have shown that females who are under oral contraceptives containing progestin have PSA present, and produce PSA in breast milk during lactating after pregnancy[2,15]and was found that Free PSA concentration reduced as the post delivery time increased [15,32]. However there is no evidence for the presence of Free PSA in serum of lactating female [33]. Ultra structural studies of breast cyst fluids, nipple aspirate fluids[NAF], breast duct cells in milk shows the ability of these cells to synthesize and secrete PSA conferring these cells may be source for the production of PSA[32, 34, 35]. Ductal lobular unit of breast is found to be a source for PSA, which is found in large quantity in extracellular fluids of NAF. Higher levels of PSA show high risk for breast cancer. Usually higher levels of PSA are seen in pre menopausal women due to higher steroid hormones circulation [35]. Various studies have shown that PSA up to 10mg/l is present in NAF [32, 17].

Gene Locus of PSA

Expression of PSA gene in female breast tissue is controlled by steroid hormones like androgens and progestin [25]. PSA coded, by 6Kbp length of DNA [47]. And belongs to Human kallikrein family and is also called hK3 [19]. The gene locus of Human kallikrein is on the chromosome 19q13.4, and is about 261558bp. It forms a largest cluster of serine proteases in human genome has 15 tandemly localized genes and has no interventions from any genes. Table 1 gives brief idea about discovery of Human kallikrein gene and their first publication [19].

Table 1: Discovery of Human Kallikrein gene

Name of the kallikrein	Genbank compliance	First Publication
KLK4	Gene AF113141	Nelson PS et al., 1999(58)
KLK5	Gene AF135028	Yousef GM et al., 1999(40)
KLK6	mRNA D78203	Yamashiro K et al., 1997(69)
	Full gene AF149289	Yousef GM et al., 1999(57)
KLK7	mRNA L33404	Hansson L et al., 1994(70)
	Full gene AF166330	Yousef GM et al., 2000(55)
KLK8	Gene AB009849	Yoshido S et al., 1998(71)
KLK9	Gene AF135026	Yousef GM et al., 2000(72)

KLK10	mRNA NM_002776	Liu XL et al., 1996 (63)
	Full gene AF055481	Luo L et al., 1998(73)
KLK11	mRNA AB012917	Yoshida S et al., 1998 (71)
	Full gene AF164623	Yousef GM et al.,2000(75)
KLK12	Gene AF135025	Yousef GM et al., 2000(60)
KLK13	Gene AF135024	Yousef GM et al., 2000(56)
KLK14	Gene AF161221	Yousef GM et al., 2001(59)
KLK15	Gene AF242195	Yousef GM et al., 2001(76)

Human kallikrein multigene locus was 1st reported in 1999 which was updated in 2000(40-42). The last memberKLK15 was cloned in the year 2001[43].

The distance between two neighbouring genes is 1.5kb [KLK1 to KLK15] to 32.5kb [KLK4 to KLK5] and is tightly packed. There are no evidences of pseudo genes presence. It is evident that minisatellites alleles are associated with malignancy. Minisatellite element is present and restricted to chromosome 19q13, whose 10 clusters are distributed along the kallikrein loci which are mainly located in promoters and enhancers, introns, untranslated regions of mRNA. Since kallikreins are expressed at both mRNA and protein levels it has a very good use in the assessment of prognosis of the malignancy [44].

Functional Activities Of   PSA

PSA present in seminal fluid are proteolytically active, it cleaves seminogellin leading to liquefaction of semen and helps to increase the sperm mobility. Anyhow the functions of   PSA in females and from other non prostatic   PSA is not clear [49].

Active   PSA will bind to protease inhibitors like ACT (α1 – antichymotrypsin) and AMG (α-2 macroglobulin),[51] and form complex and becomes inactive. Based upon the tissue in which   PSA is produced, PSA which are not exposed to protease inhibitors will exist in free form i.e. Free   PSA and are said to be proteolytically active. Sometimes   PSA would be enzymatically inactive and thus cannot interact with protease inhibitors[49].

PSA is involved in local paracrine Insulin Growth Factor pathways which will affect the growth of cells by releasing Insulin Growth Factor 1 from its binding proteins, due to this activity of   PSA it can be said that   PSA is involved in abnormal proliferation and growth of the tumour [48].

PSA is also involved in activation of matrix metallo-protease and break down of extra cellular matrix, which leads to tumour growth and metastatic growth [52].   PSA can break down extracellular glycoprotein, fibronetic, and laminin [53]. All the above functions are centred on metastasis growth factor activation.

PSA present in female breast tumour cytosol can be used for studying prognosis and there is no co relation with above mentioned functions. PSA from breast tissue have shown to produced active peptides from BRCA1 gene product, which inhibits the growth [50]. Due to lack of co relations of functions of prostatic PSA or PSA in males and PSA in female breast cancer, a lot of studies has to be carried out determine the role of non prostatic PSA or PSA produced in female breast cancer patients[46].

Expression Of PSA In Females

Steroid hormones like androgens, progesterone, activate steroid responsive elements in PSA promoter/enhancer region and regulate PSA gene in female breast tissue [26]. Breast cancer tumours positivity for PSA is not associated with positivity of oestrogen, progesterone receptor [11, 28]. The entire tumours positive for steroid hormone receptor will not produce PSA when compared to advanced cancer stage [29]. PSA is highly expressed both in mRNA and protein levels in Benign Breast Diseases [BBD] [9].

Table 2: list of different kallikrein proteins used for diagnosis and monitoring prognosis of different type of cancer.

Kallikrein protein type	Sample	Estimation method used	Type of cancer	Reference
hK2	serum	Immunoassay	Prostate and breast cancer	Rittenhouse HG et al., 1998 (62)
	Tissue	Immunohistochemistry		Rittenhouse HG et al., 1998 (62)
hK3(PSA)	Serum	Immunoassay	Prostate and breast cancer	Rittenhouse HG et al., 1998 (62)
	Tissue	Immunohistochemistry		Black MH et al., 2000 (61)
hK6	Serum	Immunoassay	Ovarian cancer	Diamandis EP et al., 2000(64)
hK10	serum	Immunoassay	Ovarian cancer	Luo LY et al., 2001(67)
hK11	serum	Immunoassay	Prostate cancer	Diamandis EP et al., 2002(68)

Role Of Molecular Forms Of PSA In Diagnosis Of Breast Cancer

There are two molecular forms of PSA:

➢ Bound PSA: PSA bound to ACT
➢ Free PSA
   Total PSA=Free PSA+ bound PSA

Free PSA is the major fraction in female breast cancer patient serum. Table 2 shows different kallikrein proteins used for diagnosis and monitoring prognosis of different type of

cancer. Using HPLC and PSA immunoassay the levels and ratio of Total PSA and Free PSA, which showed that women with BBD have high Total PSA levels than normal women and cancer patients [21]. Table 3 shows Percentage of detectable Total PSA and Free PSA at different levels [6]. Studies also shown that serum Total PSA levels are not associated with tumour size, disease stage , tumour grade, breast feeding history, relapse of cancer, lymph node involvement, type of surgery. But levels varied with women who where under chemotherapy, and without chemotherapy. High levels of Total PSA are seen in pre menopausal women. High levels of Total PSA are seen in women with ductal carcinoma than women with other types of breast carcinoma. Table 5 shows Percentage of immuno expression of PSA according to the different histopathological types of breast cancer [54].

Table 3: Percentage of detectable Total PSA and Free PSA at different levels.

	Percentage of Total PSA	Percentage of Free PSA	
Controls	29%	10%	Margot H.Black et al., 2000(6)
Pre surgical breast cancer	57%	36%	
Post surgical breast cancer	44%	11%	
Breast cysts	75%	28%	
Uterine fibroids	50%	16%	

Table 4: Diagnostic sensitivity and specificity of molecular forms of PSA.

	Diagnostic specificity	Diagnostic sensitivity	
Total PSA			Margot H.Black et al., 2000(6)
Pre surgical breast cancer/ controls	69%	57%	
Pre surgical breast cancer/breast cysts	25%	57%	
Free PSA			
Pre surgical breast cancer/ controls	90%	36%	
Pre surgical breast cancer/breast cysts	72%	36%	

Table 5: Percentage of immuno expression of PSA according to the different histopathological types of breast cancer.

Histopathological type	Percentage of PSA positive cases	
Ductal invasive	29%	Diana Narita et al., 2006 (54)
Lobular invasive	53%	
Ductal in situ	36 %	
Lobular in situ	57%	
Medullary	75%	
Mucinous	50%	
Undifferentiated	10%	
Neuro endocrine	33%	

Serum Free PSA was seen pre dominantly in about 44% cases of breast cancer patients. In most of BBD cases serum Free PSA was dominant. Free PSA is found pre dominantly in pre surgical breast cancer patients than in healthy or BBD women and also these levels reduces after surgery. Women with breast cancer and patients with BBD had no significant difference in levels of serum Free PSA levels. The levels of Free PSA is not found to be associated with tumour size, but found to be associated with histological grade, grade 3 has higher levels of Free PSA , grade 1 has no detectable Free PSA levels [21].Table 6 shows how PSA is related to different histological grades of breast cancer [54].

Table 6: Percentage of immunohistochemical expression of PSA related to different histological grades of breast cancer.(10)

Histological grade	Percentage of PSA positive cases	
G1	42%	Diana Narita et al., 2006(54)
G2	39%	
G3	34%	

Table 7: association of PSA positive tumours and age, tumour size, nodal status, ER status, and PR status.

	PSA positive cases	PSA negative cases	
Age (years)			
≤ 50	75%	25%	
≥ 50	77%	23%	
Tumour size			
≤ 2cm	79%	21%	
≥ 2cm	73%	27%	
Nodal status			
Positive	74%	26%	
Negative	76%	23%	He yu et al., 1998(45)
ER status			
Positive	76%	24%	
Negative	75%	25%	
PR status			
Positive	77%	23%	
Negative	74%	26%	

All the studies indicate that Free PSA has higher specificity and can differentiate breast cancer patients with BBD or with no breast pathology, but sensitivity of Free PSA is less. The diagnostic sensitivity of Free PSA is found to be 36%. Whereas Total PSA has higher sensitivity but specificity to differentiate breast cancer patients and BBD is less. Table 4 briefs about Diagnostic sensitivity and specificity of molecular forms of PSA [6].Due to specificity of Free PSA, it has a high potential to be used as diagnostic tool for breast cancer patients in near future, it can also be used for diagnosis of breast cancer along with the already established biomarkers.

PSA In Prognosis Of Breast Cancer

Studies have shown that PSA positive breast tumours have enhanced prognosis compared to PSA negative breast tumours. PSA status, nodal status, tumour size, is independent markers of breast cancer prognosis. Out of which PSA status is one of the favourable prognostic marker. Table 7 shows association of PSA positive tumours and age, tumour size, nodal status, ER status, and PR status [45].

Ratio of Free PSA and Total PSA can be used to classify different types of cyst like apocrine type 1 cysts, flattened type 2 cysts in gross cystic breast disease [GCBD]. It is shown that PSA concentration is increased in type 1 cysts than type 2 [36, 37, and 38]. These results confers that PSA can be used as biomarker to sub classify GCBD which can differentiate gross cysts at increased risk of breast cancer [37, 38]. PSA concentration of up to 0.5mg/l is seen in breast cyst fluid. In NAF up to 10mg/l of PSA is seen, which can also be used for risk assessment of breast cancer.

Patients with PSA positive tumours have 30-40% lower risk of relapse and death, in comparison with PSA negative tumours [22]. And thus PSA positive patients will have enhanced disease free rates and increased survival rates

Patients who were positive for estrogen receptor[ER positive] associated with PSA positive tumours have enhanced disease free rates and increased survival rates. Patients with tumour size more than 2cm associated with PSA positivity have enhanced disease free rates and increased survival rates. Since not much is known about the physiology of PSA in breast tissue it is not easy to confer PSA as a good prognostic tool. Therefore studies have to be carried out on PSA in tumour extracts to study about breast cancer prognosis [45].

CONCLUSION

In today's modern world leading causes of death in all females of all ages are heart diseases, cancer and stroke. Breast cancer is the second leading cause of cancer deaths in women today (after lung cancer) and is the most common cancer among women, excluding non melanoma skin cancers. According to the American Cancer Society, about 1.3 million women will be diagnosed with breast cancer annually worldwide about 4, 65,000 will die from the disease. Breast cancer death rates have been dropping steadily since 1990, according to the Society, because of earlier detection and better treatments. According to the American Cancer Society, in general, breast cancer rates have risen about 30% in the past 25 years in western countries.

According to the recent data from Indian Council of Medical Research (ICMR), the leading cause of death in Indian females is breast cancer; pushing the cervical cancer back to the 2nd spot. It is reported that one in 22 women in India are likely to suffer from breast cancer during her lifetime, while the figure is definitely more in America with one in eight being a victim of this deadly cancer. The most effective way to reduce death is by early detection and administration of therapy. There are several ways in which breast cancer can be diagnosed like

mammography, fine needle aspiration cytology, physical examination, and using several serum biomarkers. But all these methods mammography is not suitable and recommended to all the age groups and FNAC is painful process, available serum biomarkers sensitivity is limited, and are not suitable in early detection of breast cancer therefore a new diagnostic and prognostic biomarker is required, which has a high specificity and can be detected at very early stages. From our review we can know that Free PSA is one of the serum markers in female breast cancer patients which can be used as both diagnostic and prognostic tool. This has a high specificity and can be used to differentiate different breast diseases. PSA levels in breast tumour cytosols shows that PSA can also be used as prognostic tool. Studies show that in serum rather than bound PSA, the concentration of Free PSA is predominant and has a high specificity than Total PSA. Studies also show that patients with PSA positivity have a high survival rate and disease free. Studies have also shown that PSA expression is also associated with ER, PR, Tumour size, clinical stage and can influence prognosis of breast disease.

PSA is a marked biomarker for prostate cancer and was taught to be produced only in males, from our review we can know that PSA is also produced in females. Anyhow, in contrast to females where Free PSA is predominant, in males bound PSA is found predominantly. Since generation of PSA in female is not clear, there is a lot of scope for the studies on production of PSA in female tissue and from non prostatic sources, their gene structure, conditions required for activation of these, formation of PSA proteins.

Due to the high specificity of Free PSA in female breast cancer ,it suggest that Free PSA, either alone or with combination of several other serum biomarkers can be used for early diagnosis and study the prognosis, and risk assessment of the breast disease in females.

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