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Serum Free Prostate Specific Antigen in Indian Female Breast Cancer Patients.

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ABSTRACT

Estimating serum levels of free prostate specific antigen in Indian female breast cancer patients in order to find out significance of free prostate specific antigen in diagnosis of breast cancer in women. Hundred clinically and histopathologically confirmed female breast cancer patients of the age group of 30-65 years served as cases and hundred normal healthy females in the same age group served as controls. Free prostate specific antigen was estimated by ELISA method. Presence of Free prostate specific antigen was seen in female breast cancer patients compared to controls in which Free prostate specific antigen was absent. Levels of Free prostate specific antigen were statistically significant. Study concludes that Free prostate specific antigen can be used as serum biomarker for diagnosing breast cancer, which will be specific, precise, cost effective and suitable in rural and urban Indian scenario.

INTRODUCTION

According to National Cancer Registry Programme, India, the frequency of breast cancer prior to 25 years occurred above 50 years of age but in the present day women in the age group 25 to 40 years of age have high frequency of breast cancer. Of all feminine cancers widespread type of cancer in the majority of cities in India and 2nd most widespread in the rural areas is breast cancer which account for 25% to 32% [1].

When compared to western countries in India awareness of screening is lacking, and patients show up only when there are symptoms, so there is a need to promote screening test which are cost effective, simple and accurate [2].

Currently breast cancer diagnosis include triple assessment which includes: clinical examination, radiological investigation (mammogram, ultrasound, magnetic resonance imaging and pathological correlation (needle biopsy/FNAC), which are painful and women usually feel embarrassed to undergo these investigations , so there is a need to develop a simple accurate cost effective diagnosis test, our study may be the solution [3].

PSA as the name suggest is used as marker for detection of Prostate cancer. PSA is a serine protease discovered in 1970, has two molecular forms namely Free PSA (33KDa) and bound PSA (100KDa). PSA bound to proteinase inhibitors α1 – antichymotrypsin [PSA-ACT] is known as bound PSA. Total PSA is Free and Bound PSA together [4, 5]. In 1989 PSA was discovered in female fibrocystic breast tissue.[6] PSA is proved to be not Prostate gland specific.[7] Its presence in non prostate tissues or glands

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like peri urethral gland, breast tumours, breast cysts, nipple aspirate fluid have been proved [8, 9, 10-13, 14, 15, 16-18]. Molecular mass of both Prostate and breast tumour were same.[6] bound PSA is predominant in males compared to females where Free PSA is predominant[19]. Diagnostic specificity of Free PSA is higher than Total PSA in female breast cancer patients [20].

The main aim of this study is to find out potential of Free PSA as diagnostic biomarker in Indian females

MATERIALS AND METHODS

Study consisted of 100 female breast cancer patients and 100 apparently healthy women who are age matched with them were selected. The cancer patients were from Manipal Super Specialty Hospital and City Cancer Center, Vijayawada. Controls were randomly selected women attending the above hospital. The duration of study was from 2011-2012.

Inclusion criteria

Freshly diagnosed female breast cancer Patients and controls in the age group30-60 years.

Exclusion criteria

Female Patients or controls suffering from tuberculosis, rheumatic fever, hemolytic anemia, hypertension , diabetes mellitus, hepatitis, jaundice, pregnancy or breast feeding, bone diseases, pancreatic disease, congestive cardiac failure, myocardial infarction, ulcerative colitis, other malignancies and patients who had already received or were under treatment for malignancy were excluded from study. Clinical investigations and questionnaires formed the basis of enquiry.

Collection of blood

Under strictly aseptic conditions 5ml of fasting venous blood was drawn from median cubital /basilic vein into BD red capped plain Vacutainers. Vacutainers were made to stand for 10 min at room temperature to allow clotting. Later centrifuged at 3000rpm for 10 minutes using Remi8RC centrifuge. Serum was separated and test was carried out on the same day.

Estimation of Free PSA

ELISA method

Principle

Quantitative estimation of Free PSA concentration in human serum by micro plate immunoenzymometric assay was carried out using Accu-Bind ELISA micro wells from Monobind Inc. (USA). Free PSA calibrator, patient or control serum is first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labeled antibodies are added which form sandwich complex which binds with streptavidin coated wells.

$$^{Enz}Ab_{(P)} + AgfPSA + {}^{Btn}Ab_{(m)} \Longleftrightarrow {}^{Enz}Ab_{(P)}\text{-}AgfPSA\text{-}{}^{Btn}Ab_{(m)}$$

$$^{Enz}Ab_{(P)}\text{-}AgfPSA\text{-}{}^{Btn}Ab_{(m)} + \text{streptavidin}_{cw} \Longrightarrow \text{immobilized complex}$$

Enzyme –fPSA antibody bound conjugate is separated from unbound Enzyme –fPSA conjugate by decantation. Activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known fPSA levels permits construction of a dose response curve. By comparing dose response curve with unknown patient or control serum fPSA activity can be correlated with fPSA concentration. ELISA reader of MERCK Company was used to take absorbance of the wells.

$^{Enz}Ab_{(P)} \Longrightarrow$ enzyme labeled antibody

AgfPSA $\Longrightarrow$ native antigen

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$^{Btn}Ab_{(m)}$ ⟹ biotinylated antibody

$^{Enz}Ab_{(P)}$-AgfPSA-$^{Btn}Ab_{(m)}$ ⟹ antigen antibodies complex

Streptavidin $_{cw}$ ⟹ streptavidin immobilized on well.

Immobilized complex ⟹ complex bound to solid surface.

Statistical analysis was carried out by using **SPSS 16.0 software.** Using independent sample test with P< 0.05 SIGNIFICANT.

RESULTS

Study showed presence of Free PSA in female breast cancer patients whereas complete absent or undetectable level of Free PSA in controls. [Graph1]. Statistical analysis showed significant value of P<0.00 [Table 1]. This Study proved Free PSA can be used as diagnostic tool in diagnosing breast cancer in Indian women.

Soft ware used: SPSS 16.0, Method: Independent Samples Test

Graph 1: levels of Free PSA in controls and cases.

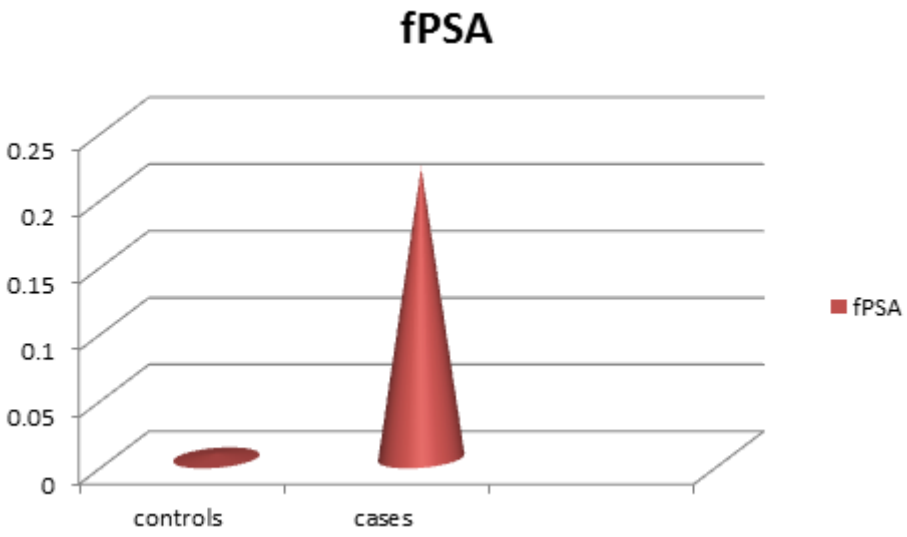

Table 1: Group Statistics

Free PSA	N	Mean	Std. Deviation	Std. Error Mean	p
Controls	100	.0039	.01761	.00176	<0.00
Cases	100	.2178	.13394	.01339	

P<0.05 SIGNIFICANT

DISCUSSION

Prostate specific antigen is been used in detection and post surgical supervision of Prostate cancer [21]. Molecular forms of PSA have been successfully used in medical practice to differentiate prostate cancer and benign prostatic hyperplasia [22]. Larger portion of PSA complexed to ACT is seen in patients' serum of prostate cancer [23].

Studies have shown that Free PSA forms a major fraction in the serum of breast cancer patients. [24] Free PSA and PSA-ACT show to be in more or less in equivalent proportions in breast cyst fluid [25].

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Breast tumors generate an endopeptidase which causes a posttranslational alteration of PSA formed by the breast; hence prevent complex formation with ACT and raising the fraction of free PSA [26]. However extent of difference in production of PSA molecular forms in benign or malignant prostatic or mammary tissue is not yet understandable[24].

Various studies are currently being carried out to discover new serological markers of breast tumors, like carcino embryonic antigen, carbohydrate antigen 15.3, tissue polypeptide-specific antigen, and mammary serum antigen, but diagnostic sensitivity is less compared to Free PSA [27–29].

High degree specificity of Free PSA make it promising budding serum marker either alone or in combination with additional markers. Additional studies with larger population of patients are necessary for the assessment of molecular forms of PSA with regard to individual parameters such as threat of reversion or metastasis. In conclusion, the physiological method behind the free PSA raise in serum of breast cancer patients with respect to tumor progression must be further investigated.

CONCLUSION

Study concludes that Free PSA can be used as serum biomarker for diagnosing breast cancer, which will be specific, precise, cost effective, and suitable in rural and urban Indian scenario. Because of the simplicity of the test it can be carried out in any small set up which make it accessible for all class of people. Investigating Free PSA alone or with combination with simple biochemical marker may be one of the upcoming diagnosing means.

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