IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 5 Ser.8 (May. 2020), PP 57-64
www.iosrjournals.org

Prostate Specific Antigen-A Further Play in Carcinoma Breast

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Abstract
OBJECTIVE - To identify the diagnostic role of PSA in carcinoma breast and to evaluate PSA as a valuable tool for prognosis of breast cancer.
MATERIAL AND METHODS - The study was conducted on 50 female patients with carcinoma breast and 50 normal females at RIMS, Ranchi Jharkhand.
RESULTS- We demonstrated that estimation of PSA in carcinoma breast can be regarded as a marker for diagnosis of breast cancer. Although, free PSA as the predominant molecular form is highly specific and statistically significant (p< 0.005) for breast cancer.
CONCLUSION- Hence to conclude from my study is that there is definitely a role of PSA in carcinoma breast and must be recommended to all patients with carcinoma breast.
Key Words-*PSA,Carcinoma breast*

Date of Submission: 06-05-2020 Date of Acceptance: 19-05-2020

I. Introduction

In India breast cancer is the second most common cancer in women next to cervical cancer. According to National Cancer Registry Programme in India the frequency of breast cancer prior to 25 years occurred above 50 years of age, but in present day women in the age group of 25-40 years of age has high frequency of breast cancer. Highest incidence of breast cancer is seen in the age 45-49 years. Multiple factors are associated with an increased risk of developing breast cancer; these can be genetic, environmental and histological factors.

Tumour markers are used for detection of risk, population screening, diagnosis, staging and prognosis of cancers. It can also predict the response to the therapy, the presence of occult metastasis and monitor the course of disease. Various tumour markers have been studied singly or in combination in carcinoma breast. Most of these are high on running costs, in terms of laboratory infrastructure.

Prostate specific antigen (PSA) is a valuable tumour marker for the diagnosis and management of prostate carcinoma. Currently, PSA is a highly valuable marker for prostate cancer screening, diagnosis and postsurgical monitoring of prostate cancer patients, as well as for the detection of micrometastasis [1]. It was believed that PSA was produced exclusively by the epithelial cells of the prostate gland. It is widely accepted that PSA is not prostate specific [2]. PSA has been shown to be expressed in many forms of female tissues. The breast is a major female organ able to produce PSA. It is detected in both normal and abnormal breast tissues, as well as in breast fluids including milk, nipple aspirate, and cyst fluid. Androgen and progesterone, via their receptors, regulate the production of PSA in breast tissues. Clinical studies demonstrate that PSA in breast cancer is associated with the expression of oestrogen receptor and progesterone receptor. PSA is produced by the majority of breast tumours and may be a favourable indicator of prognosis in breast cancer [3, 4].

PSA is a 33kDa glycoprotein containing 237 amino acids, 4 carbohydrate side chains and multiple disulphide bonds which is homologous with the proteases of the kallikrein family and hence called human glandular kallikrein-3 (hk-3). 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 of α-1 antichymotrypsin [PSA-ACT] is known as bound PSA. Total PSA is free and bound PSA together [5, 6].

The gene encoding PSA has been sequenced and localized to chromosome 19. It is similar to the kallikrein -1 gene with 82 % homology. PSA exists in two forms in the blood circulation. Most PSA is complexed with the protease inhibitors α1-antichymotrypsin (ACT) (molecular weight 1, 00,000 Da) and α2-macroglobulin (AMG) and a minor component is free PSA (molecular weight 28,430 Da). Most immunoassays measure both free and ACT-complexed PSA but not PSA-AMG, which is sterically inhibited. The metabolic clearance rate of PSA follows a two-compartment model, with initial half-lives of 1.2 and 0.75 hours for free PSA and total PSA and subsequent half-lives of 22 and 33 hours.

In 1989, PSA expression was first discovered through immunohistochemical reactions in some apocrine foci of female fibrocystic breast tissue as well as in breast cancer (BC), casting doubt on the specificity of this kallikrein only for prostatic epithelium [7].

Yu H, Berkel H. studied Prostate-specific antigen (PSA) in women and stated that breast is a major female organ able to produce PSA. PSA is detected in both normal and abnormal breast tissues as well as in various breast fluids including milk, nipple aspirate, and cyst fluid. They also stated that high concentration of PSA is found in amniotic fluid and the levels change with gestational age [8].

Thus the present study is designed to evaluate the levels of serum total and free PSA in carcinoma breast and to assess their role and significance. PSA can also be used as a diagnostic and prognostic marker of breast cancer in women, therefore helping secondary prevention of breast cancer.

II. Aims And Objectives

The main aim of this study is to evaluate the levels of Total and Free PSA in carcinoma breast in females and normal females.

To identify the diagnostic role of Total and Free PSA in carcinoma breast.

III. Material And Methods

The study was an observational study conducted on female patients with carcinoma breast admitted in surgical ward at Rajendra Institute of Medical Sciences, Ranchi during the period January 2018-October 2019

SAMPLE SIZE: A total of 100 subjects were taken.

GROUP A- Includes 50 patients which were diagnosed with carcinoma breast by tissue diagnosis.

GROUP B- Includes 50 controls who were of the same age matched normal healthy individuals.

INCLUSION CRIETERIA

All female diagnosed clinically and histopathologically with carcinoma breast of any age group before treatment and surgery.

EXCLUSION CRIETERIA

Female patients suffering from:

Tuberculosis, Rheumatic fever, Haemolytic anaemia, Hypertension, Diabetes mellitus, Hepatitis, Jaundice, Pancreatic diseases, Pregnancy or breast feeding, Bone diseases, Cardiac failure, Myocardial infarction, Ulcerative colitis and other malignancies.

REFERENCE VALUES

NORMAL FEMALES - < 0.01 ng/ml [9].

IV. Methodology

Blood was obtained with written consent of each cases and controls and a proper questionnaire was provided. Clinical history was taken from the subjects and examination findings were noted.

Proper physical examination and history was taken along with questionnaire was provided with written consent.

BIOCHEMICAL EXAMINATION

1. Total PSA 2. Free PSA

METHOD OF SAMPLE COLLECTION

- Patients were instructed for the procedure to be undertaken and to remain calm and compose.
- Following safety measures were followed while collecting blood samples:

•Wearing gloves after handling blood.

•Changing gloves after handling of each patient or when contaminated.

•Washing hands frequently.

•Disposal of items in appropriate containers.

•Disposal of needles immediately upon removal from patients.

•Cleaning up any blood spills with a freshly made 10% bleach disinfectant.

Needles of 20 or 22 G Size were used for blood samples. Needles were put in disposable unit immediately after their use. 70% isopropyl alcohol was used in cotton swab to wipe and sterilize the skin.

PROCEDURE

The antecubital vein of the arm was preferred and used most frequently for collection of blood. First the surface was palpated and the path vein was traced with index figure. Arteries were identified and avoided as they pulsate, are more elastic and having a thick wall. Once the preferred site was selected, gloves were put on. Venepuncture site was cleaned with alcohol preparation, cleansing in a circular fashion. The site was allowed to dry.

The tourniquet was applied 3-4 inches above the selected puncture site. Precaution was taken not to apply tourniquet too tightly. Venepuncture performed with patients arm rested in a comfortable position. Tourniquet was removed as soon as the blood appeared in the syringe. About 5-6 ml of blood was withdrawn. When blood has been collected, a cotton ball or gauze was placed over the site and the needle withdrawn in a smooth and cautious manner so as not to bruise the vein or skin. After withdrawing the needle, gentle pressure was applied to the cotton ball over the puncture site. The patient was asked to apply pressure for 3-5 minutes to prevent oozing of blood from puncture site. Then blood samples were put it appropriate vial.

The serum sample blood was allowed to clot by placing in the rack at room temperature for at least 30 minutes. Then it was centrifuged at 3000 rpm for 10 minutes and serum was separated. The serum samples were stored at -80ºC. The clear serum was then analysed for the estimation of serum Total PSA and Free PSA.

ANALYSIS

Estimation of Prostate-specific Antigen (PSA) by ABOTT (Architect Plus i 1000SR)

The biochemical parameters undertaken for the estimation of serum total PSA and free PSA was determined in a fully automated in a fully automated ABOTT (Architect Plus i 1000SR)

The Access Hybritech PSA assay is a two-site immunoenzymatic "sandwich" assay.

A sample is added to a reaction vessel with mouse monoclonal anti-PSA alkaline phosphatase conjugate and paramagnetic particles coated with a second mouse monoclonal anti-PSA antibody. The PSA in the sample binds to the immobilized monoclonal anti-PSA on the solid phase while, at the same time, the monoclonal anti-PSA conjugate reacts with a different antigenic site on the sample PSA. Separation in a magnetic field and washing removes material not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos** 530 is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is proportional to the concentration of PSA in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

Detection limit: 0.008 ng/ml

The ARCHITECT Free PSA assay is a two-step immunoassay to determine the presence of free PSA in human serum, using ChemiluminescentMicroparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample and anti-free PSA coated paramagnetic microparticles are combined. Free PSA present in the sample binds to the anti-free PSA coated microparticles. After washing, anti-PSA acridinium-labelled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of free PSA in the sample and the RLUs detected by the ARCHITECT i* optical system. The ARCHITECT Free PSA assay is intended to be used in conjunction with the ARCHITECT.

STATISTICAL METHOD AND DATA ANALYSIS

Statistical analysis was done using SPSS software. The data was represented by counts, percentage and mean ± standard deviation. Statistical analysis of the biochemical tests of Total PSA and Free PSA was done by unpaired t-test to compare the parameters in carcinoma breast cases and control.

A P value of <0.05 was considered statistically significant.

V. Observation And Results

The present study is an observational study comprising of 100 patients including 50 cases of carcinoma breast (Group A) and 50 cases of control (Group B) in Rajendra Institute of Medical Sciences, Ranchi during the period January 2018-October 2019.

The general characteristics of the study populations are stated below:

TABLE 1 – Distribution of cases (Group A) according to Age

Age Distribution of Cases (Group A)		
Age	**Cases (n=50)**	**Percentage%**
20-45 Years	24	48
46-65 Years	25	50
>65 Years	1	2
Total	**50**	**100**

Table 1 shows the distribution of cases (Group A) according to age in carcinoma breast.
Minimum age was 23 years and maximum was 73 years. Mean age was found to be 45.70 ±10.929 years.
Maximum (25) number of cases were found in the age group 46-65 years and minimum (01) number of cases were found in the age group >65 years.

TABLE 2 – Distribution of Control (Group B) according to Age

Age Distribution of control (Group B)		
Age	**Cases (n=50)**	**Percentage %**
20-45 Years	32	64
46-65 Years	17	34
>65 Years	01	02
Total	**50**	**100**

Table 2 shows the distribution of control (Group B) according to age.
Minimum age was 30 years and maximum was 73 years. Mean was found to be 45.50 ± 10.053 years.
Maximum (32) number of cases were found in the age group 20-45 years and minimum (01) number of cases were found in the age group >65 years.

TABLE 3 - Distribution of Total PSA value according to Age in Cases (Group A)

Variables	**Total PSA Level (ng/ml)**					
AGE	**0.008**	**0.158**	**0.026**	**0.093**	**0.022**	**TOTAL**
20-45 Years	22	0	01	01	0	**24**
46-65 Years	23	01	0	0	01	**25**
>65 Years	01	0	0	0	0	**01**
TOTAL	**46**	**01**	**01**	**01**	**01**	**50**

Table 3 shows the distribution of Total PSA according to age in cases (Group A).
The value of Total PSA in cases (Group A) wasfound in the range of 0.008- 0.158 ng/ml.
Minimum (01) case wasseen in the age group of >65 yearswhile the maximum (24) cases werefound in the age group of 20-45 years.
Minimum (0.008ng/ml) value of Total PSA wasfound in maximum (46) number of cases and maximum (0.158 ng/ml) valuewasfoundonly in one case.

TABLE 4 - Distribution of Total PSA valueaccording to age in Control (Group B)

Variables	**Total PSA Level (ng/ml)**				
AGE	**0.008**	**0.021**	**0.018**	**0.031**	**TOTAL**
20-45 Years	28	02	01	01	**32**
46-65 Years	17	0	0	0	**17**
>65 Years	01	0	0	0	**01**
TOTAL	**46**	**02**	**01**	**01**	**50**

Table 4 shows the distribution of Total PSA according to age in control (Group B).
The value of Total PSA in control (Group B) wasfound in the range of 0.008-0.31 ng/ml.
Minimum (01) case wasseen in the age group of >65 yearswhile the maximum (32) cases werefound in the age group of 20-45 years.
Minimum (0.008 ng/ml) value of Total PSA wasfound in maximum (46) number of cases and maximum (0.31 ng/ml) valuewasfoundonly in one case.

TABLE 5 - Distribution of Free PSA value according to age in Cases (Group A)

Variables	Free PSA Level (ng/ml)									
AGE	**0.001**	**0.002**	**0.039**	**0.006**	**0.003**	**0.005**	**0.037**	**0.23**	**0.004**	**TOTAL**
20-45 Years	16	05	0	02	0	0	01	0	0	**24**
46-65 Years	11	02	01	0	04	02	0	01	04	**25**
>65 Years	01	0	0	0	0	0	0	0	0	**01**
TOTAL	**28**	**07**	**01**	**02**	**04**	**02**	**01**	**01**	**04**	**50**

Table 5 shows the distribution of Free PSA according to age in cases (Group A).
The value of Free PSA in cases (Group A) was found in the range of 0.001-0.23 ng/ml.
Minimum (01) case was seen in the age group of >65 years while the maximum (25) cases were found in the age group of 46-65 years.
Minimum (0.001ng/ml) value of Free PSA was found in maximum (28) number of cases and maximum (0.23 ng/ml) value was found only in one case.

TABLE 6 - Distribution of Free PSA value according to age in control (Group B)

Variables	Free PSA Level (ng/ml)									
AGE	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**	**0.006**	**0.016**	**0.008**	**0.007**	**TOTAL**
20-45 Years	13	04	02	02	04	04	01	02	0	**32**
46-65 Years	07	02	02	02	01	02	0	0	01	**17**
>65 Years	01	0	0	0	0	0	0	0	0	**01**
TOTAL	**21**	**06**	**04**	**04**	**05**	**06**	**01**	**02**	**01**	**50**

Table 6 shows the distribution of Free PSA according to age in control (Group B).
The value of Free PSA in control (Group B) wasfound in the range of 0.001-0.016 ng/ml.
Minimum (01) case wasseen in the age group of >65 yearswhile the maximum (32) cases werefound in the age group of 20-45 years.
Minimum (0.001 ng/ml) value of Free PSA wasfound in maximum (21) number of cases and maximum (0.016 ng/ml) valuewasfoundonly in one case.

Table 7-Comparison of Total PSA meanvalue in cases (Group A) and control (GroupB)

Comparison of Total PSA Mean Value in cases (Group A) and control (Group B)		
Variables	**Cases**	**Control**
Mean	0.0091	0.00382
Std. Deviation	0.004062	0.007782
P Value < 0.001		

Table 7 shows the comparison of Total PSA meanvalue in cases (Group A) and control (Group B)
The meanwasfound to be 0.0091 and 0.00382 in cases (Group A) andcontrol (Group B) respectively.
The standard deviation of Total PSA value in cases (Group A) and control (Group B) was 0.004062 and 0.007782 respectively.
P valuewas< 0.001 and highlysignificant.

Table 8 - Comparison of Free PSA meanvalue in cases (Group A) and control (Group B)

Comparison of Free PSA Mean Value in cases (Group A) and control (Group B)		
Variables	**Cases**	**Control**
Mean	0.0382	0.322
Std. Deviation	0.07782	0.002859
P VALUE < 0.05		

Table 8 shows the comparison of Free PSA meanvalue in cases (Group A) and control (Group B)
The meanwasfound to be 0.0382 and 0.322 in cases (Group A) and control (Group B) respectively.
The standard deviation of Free PSA value in cases (Group A) and control (Group B) were 0.07782 and 0.002859 respectively.
P valuewas< 0.05 and wassignificant.

VI. Discussion

The present studywas an observationalstudycomprising of 100 patients including 50 cases of carcinomabreast (Group A) and 50 cases as controls (Group B) in RajendraInstitute of Medical Sciences, Ranchi.

Breast cancer is a leadingcause of morbidity and mortality in females of developedcountries and is the most commonmalignancyamong North American women [10]. Itisestimatedthat by the year 2000, 500,000 womenworldwidewill die from breast cancer. Currently, the most effectiveway to minimizemorbidity and mortality from breast cancer is by earlydiagnosis and administration of therapy [11]. It isthushighlydesirable to devise new methods of earlydiagnosis. Mammography is the most sensitive and specific screening modality for breast cancer; however, data presentlyavailable do not warrant a universal recommendation for mammography for all women [12].

AGE

In the present study of 50 cases (Group A) of carcinomabreast it wasfoundthat the minimum age of carcinomabreastwasseen in 23 years of age and maximum in 73 years. Mean age wasfound to be 45.70 ± 10.929 years. Maximum (25) number of cases werefound in the age group 46-65 years and minimum (01) number of cases werefound in the age group>65 years.

In the controlstudy of 50 cases (Group B) the minimum age was 30 years and maximumwas 73 years. Mean wasfound to be 45.50 ±10.053 years.

Maximum (32) number of cases werefound in the age group 20-45 years and minimum (01) number of cases werefound in the age group>65 years.

Similar studies done by Soumen et al (2011) [13] alsoshowedthat the minimum age of carcinomabreastwas 25 years and maximum age was 66 years. The mean age of carcinomabreastwas 46.6 ± 9.55 years.

Fawzi et al (2013) [14] intheirstudyalsoshowedthat the mean age for carcinomabreast patients was 49.1 ± 11.1 years and 48.1 ± 10.7 years in cases and controlrespectivelywhichwasverymuchsimilar to mystudy.

Syed et al (2015) [15] in theirstudyalsofoundthatcarcinomabreastwasseen in the age group of 36 years to 85 years and 28 years to 63 years in cases and controlrespectivelywhichwasalsosimilarto mystudy. The mean age wasfound to be 61 ±3 years and 38 ±4 years for cases and controlwhichwasalsomore or lesssimilar to mystudy.

Elteza et al (2017) [16] alsoshowed in theirstudythat the mean for cases and controlwere 42.74 ± 10.37 years and 43.34 ± 10.95 yearswhichwasalmostsimilar to mystudy.

TOTAL PSA

The value of Total PSA in cases (Group A) wasfound to be in the range of 0.008-0.158 ng/ml and the value of Total PSA in control (Group B) wasfound in the range of 0.008-0.31ng/ml. The meanwasfound to be 0.009 ng/ml and 0.00382 ng/ml in cases (Group A) and control (Group B) respectively. The standard deviation of Total PSA value in cases (Group A) and control (Group B) was 0.004062 and 0.007782 respectively. The relationship of Total PSA between cases (Group A) and control (Group B) wasstatisticallysignificant (<0.001).

Yu H et al (1998) [17] in theirstudydefined the value of Total PSA in the range of 0-8.8 ng/ml in

carcinomabreast and 0-0.84 ng/ml in controls. This was not similar to mystudy.

Margot H. Black et al (2000) [18] in theirstudyalsodefined the value of Total PSA in the range of 0-0.8153 ng/ml in cases and 0-0.0055 ng/ml in controlwhichwassimilar to mystudy.

Hayder M. Abdulnabi et al (2011) [19] in theirstudyalsodefined the value of Total PSA in the range of 1.38-5.00 ng/ml in cases and 0.0-0.0893 ng/ml in controlwhichwassimilar to mystudy.

Fawzi C. Mashkoor et al (2013) [14] found out the value of Total PSA in the range of 0.10-3.62 ng/ml in cases and 0-1.3 ng/ml in controlrespectively. The meanwas 1.373 ng/ml and 0.517 ng/ml respectively. This findingwassimilar to mystudy. The standard deviation wasfound to be 0.855 and 0.381 in cases and control in theirstudywhichhoweverwas not similar to mystudy.

B Sharma et al (2014) [20] found the value of Total PSA as<0.04 ng/ml in cases and controlwhichwas not similar to mystudy.

Syed H Emami et al (2015) [15] in theirstudydefined the value of Total PSA of 0.77 ± 0.01ng/ml in cases and 0.17 ± 0.016 ng/ml in control. My studywasverymuchsimilar to thisstudy.

Elteza T Jahir et al (2017) [16] found the mean of Total PSA to be 1.037 ng/ml and 0.129 ng/ml in cases and controlrespectivelywhichwassimilar to mystudy.

FREE PSA

The value of Free PSA in cases (Group A) wasfound in the range of 0.001- 0.23 ng/ml and
the value of Free PSA in control (Group B) wasfound in the range of 0.001-0.016 ng/ml.

The meanwasfound to be 0.0382 ng/ml and 0.0322 ng/ml in cases (Group A) and control (Group B) respectively. The standard deviation of Free PSA value in cases (Group A) and control (Group B) was 0.07782 and 0.00859 respectively. The relationship of Free PSA between cases (Group A) and control (Group B) wasstatisticallysignificant (<0.05).

Margot H. Black et al (2000) [18] in theirstudydefined the value of Free PSA in the range of 0-0.02451ng/ml and 0-0.0006 ng/ml in cases and controlwhichwassimilar to mystudy.

Hayder M. Abdulnabi et al (2011) [19] defined in theirstudy the mean of Free PSA to be 2.3493 ng/ml and 0.668 ng/ml in cases and controlrespectivelywhichwas not similar to cases but wassimilar to control in mystudy. The standard deviation was 1.3800 and 0.36806 in cases and controlrespectivelywhichwas not similar to mystudy.

Fawzi C. Mashkoor et al (2013) [14] defined the range of Free PSA to be 0-2.71ng/ml and 0-0.55 ng/ml in cases and controlrespectivelywhichwas not similar to mystudy. The mean of Free PSA wasfound to be 0.828 ng/ml and 0.148ng/ml in cases and controlrespectivelywhichwasalso not similar to mystudy. Standard deviation wasfound to be 0.61 and 0.128 respectively.

Pavithra V et al (2014) [21] found the mean of Free PSA to be 0.2178 ng/ml and 0.0039 ng/ml in cases and controlrespectively and standard deviation to be 0.133394 and 0.01761 respectivelywhichwas not similar to cases but wassimilar to control in mystudy.

Syed H Emami et al (2015) [15] in theirstudydefined the value of Free PSA to be 0.39 ± 0.12 ng/ml in cases and 0.009 ± 0.03 ng/ml in control. My studywasverymuchsimilar to thisstudy.

Elteza T Jahir et al (2017) [16] found the meanof Free PSA to be 0.864 ng/ml and 0.088 ng/ml in cases and controlrespectivelywhichwas not similar to mystudy.

VII. Conclusion

Hence to conclude from mystudy is thatthere is definitely a role of Total and Free PSA in carcinomabreast and must berecommended to all patients.Finally to concludethatwe have demonstrated the Total PSA and Free PSA as the predominantmolecular form of serological PSA in a significant proportion of breast cancer patients. Althoughfree PSA as the predominantmolecular form is highlyspecific for breast cancer, itsclinicalutility is limited at this time due to lowsensitivity. Given the heterogeneity of the population of breastcancer patients, additional studies with largercohorts of patients arerequired for the examination of molecular forms of PSA with regard to individual parameters such as risk of relapse or metastasis. Finally, the physiologicalmechanismbehind the free PSA increase in breast cancer and itsramifications with respect to tumour progression shouldbefurtherinvestigated.

VIII. Recommendation

There is association between serum level of Total PSA andFree PSA in carcinomabreast and regarded as a marker for diagnosis of breast cancer. Weadvise to send every patient with breastlump for Total and Free PSA estimation to detect cancer. My study shows that serum level of Total PSA andFree PSA is a diagnostic and usefultumour marker like in diagnosis of prostatecarcinoma and alsobeused as a prognostic factor with carcinomabreastlikeprogesterone and oestrogen receptor, but weneedfurther studies and a muchlargerpopulation size. A follow up study with monitoring of tumour marker levels post-treatmentwould have beenbetter for

establishing the prognosticvalue of PSA in breastcarcinoma. PSA estimation in the breasttissuewouldalso have been more specific.

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DR. BELA ROSE EKKA, et. al. "Prostate Specific Antigen-A Further Play in Carcinoma Breast ."*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(5), 2020, pp. 57-64.