Development of an Immunoradiometric Assay for Determination of PSA in Benign and Malignant Brest Tumors

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Abstract

Total prostate specific antigen (t-PSA) levels were measured in sera and tissues of three groups of patients with breast tumors using immunoradiometric assay (IRMA). Group I consisted of 29 women suffering from benign breast tumors. Group II and III consisted of 21 and 27 women suffering from pre- and postmenopausal malignant breast tumors. In addition to that, another group of 32 women were used as control. The results obtained revealed that the levels of serum t-PSA in group I was increased significantly ($P< 0.0001$) whereas in group II and III, the levels of serum t-PSA were slightly significant ($P< 0.05$). An immunoradiometric assay procedure was developed for the determination of t-PSA in the three groups. Total PSA (t-PSA) level in tissues of patients with group I was significantly higher than in group II and III ($p< 0.001$) whereas the level of t-PSA in tissues of patients with group II and III were no significant ($P< 0.1$). The results obtained revealed higher incidence of t-PSA in group I than those in group II and III.

الخلاصة

تم قياس مستوى المستضد النوعي البروستاتي الكلي (tPSA) في أمصال دم وانسجة ثلاثة مجاميع من مرضى أورام الثدي باستخدام الاختبار المناعي الاشعاعي المتري (IRMA). تتألف المجموعة I من 29 سيدة تعاني من أورام الثدي الحميدة، فيما تتكون الجموعة II و III من 21 و 27 سيدة تعاني من أورام الثدي الخبيثة بأعمار قبل وبعد انقطاع الطمث على التوالي، فضلا عن استخدام مجموعة تتكون من 32 سيدة من الاصحاء كمجموعة سيطرة.

دلت النتائج الى وجود زيادة معنوية في مستوى (tPSA) في أمصال دم المجموعة الاولى (P <0.0001)، في حين ان هناك زيادة اقل (P <0.05) في كلتا المجموعتين II و III.

تم تطوير طريقة الاختبار المناعي الاشعاعي المتري (IRMA) لتعيين مستوى (tPSA)في انسجة المجاميع الثلاثة I و II و III.

دلت النتائج الى وجود زيادة معنوية في مستوى (tPSA) في أنسجة المجموعة الاولى (P<0. 001) مقارنة مع كلتا المجموعتين II و III. ، في حين لايوجد تأثير معنوي (P <0.1) بين المجموعتين II و III.

تشير جميع النتائج التي تم الحصول عليها الى حدوث زيادة في مستوى tPSA في المجموعة I مقارنة بالمجموعتين II و III.

Introduction

Prostate specific antigen is widely used as the most important tumor marker for early detection, staging and monitoring men with prostate cancer [1]. Despite the original notion that PSA was a prostatic tissue specific marker, its now well accepted that PSA can be found in many nonprostatic tissues and fluids [2], like the serum of breast tumors [3] and tissue [4].

Black *et al* [3] showed that serum t-PSA levels in women with breast cancer were significantly higher than healthy women, and in women with breast cysts were significantly higher than women with breast cancer. But another study has shown serum t-PSA concentrations to be significantly lower in women with breast cancer than women with benign breast disease and no significant difference was found between cancer patients and normal women [5]. In another study, no significant difference in serum t-PSA levels was found between breast cancer patients and women with benign breast disease or healthy women [6].

According to these perturbation studies, further work is required to investigate which of these studies should be supported through development of immunoradiometric assay for determination of PSA and the finding of the optimum reaction conditions for ^{125}I- anti total PSA antibody with PSA in benign and malignant breast tumor homogenates.

Chemicals and Instruments

Chemicals

All common laboratory chemicals and reagents were of analar grade and were used with out further purification Tris (hydroxy methyl) aminomethan, Bovine serum albumin, PEG M.W 6,000, were obtained from Fluka.

EDTA (disodium salt), Na, K-tartarate, $CuSO_4$. $5H_2O$, Na_2CO_3, NaOH, HCl, , Folin Ciocalteau reagent were obtained from BDH

Immunoradiometric assay Kit for total- PSA was purchased from Immunotech- Beckman Coulter Company Czech Repoblic.

Instruments

The instruments used in this work were:

LKB gamma counter type 1270-rack gamma II, LKB spectrophotometer ultraspace type 4050, Pye-unicom pH meter, Cooling centrifuge type Hettich (5000 rpm), Memmert incubator, Memmert water bath, Orbital shaker (Lab-lin orbit invarian-shaker), Magnetic stirrer hotplate (Staurt scientific).

Samples and Solutions

Samples

Patients

One group of benign breast tumor patients and two groups of patients with malignant breast tumors were included in this study.

Group I contained 29 patients with benign breast tumor. Group II consisted of 21 premenopausal patients with breast cancer. Group III comprised 27 postmenopausal patients with breast cancer. In addition group I**V** contained 32 of matched healthy subjects were also included.

All patients were admitted for treatment to AL-Kademyah Teaching Hospital, Medical City of Baghdad Teaching Hospital and Al-Yermuk Hospital.

They were histologically proven, newly diagnosed and not underwent any type of therapy. Patients suffered from any disease that may interfere with this study were excluded.

Collection of specimens

The tumor tissues were surgically removed from breast tumor

patients by either mastectomy or lumpectomy. The specimens were cut off and immediately immersed in ice-cold isotonic saline solution. They were collected individually in plastic receptacle and stored at –20 ^{o}C until homogenization.

Preparation of breast tumor tissue homogenates

The frozen tissues were weighed, sliced finely and scalpels in petri dish standing on ice bath, the slices were thawed and further minced scissors. The minced tissues were homogenized at 4 ^{o}C in Tris buffer (TES buffer pH 7.4) with a ratio of 1: 5 (weight: volume) using a manual homogenizer. The homogenate was filtered through ten layers of nylon gauze in order to eliminate fiber connective tissues. The homogenate was centrifuged at 1000 xg for 15 minute at 4 ^{o}C in order to precipitate the remaining intact cells and the intact nucleus. The sediment was discarded and the supernatant was taken and the total protein content in breast tumor tissue homogenates were measured by the method of Lowry et al [7]. The supernatant was divided in aliquots and freezed at -20 ^{o}C until use.

Preparation of blood samples

Five milliliters of blood samples were obtained from patients undergoing mastectomy or lumpectomy by venipuncture just before surgery. Thirty-two physically normal age matched volunteers were used as controls. Blood samples were left for 20 minutes at room temperature. After coagulation, sera were separated by centrifugation at 2000 xg for 10 min. Sera were aspirated and stored in capped sterilized tubes at -20°C until time of analysis.

Solutions

Tris buffer sucrose and EDTA (TES) pH 7.4: TES buffer (0.05M, pH7.4). This buffer was prepared by dissolving 0.6075g of tris (hydroxymethyl) aminomethane, 0.1816g of EDTA and 8.5575g of sucrose in distilled water. Then the pH was adjusted to 7.4 using HCL (0.1N). The volume was made up to 100ml with distilled water.

Evaluation of total PSA in sera and tissues of breast tumor Patients

Determination of total PSA level in sera of patients with breast tumors and controls

Total PSA levels were measured in sera of benign and malignant breast tumors patients and healthy individuals were used as controls by immunoradiometric assay (IRMA). The assay protocol was described as mentioned in the total PSA IRMA kit from Immunotech- Czech Republic.

Calculations

The mean net count for each group of tubes was counted in a gamma counter for one minute, and the radioactivity of each tube refers to the amount of bound PSA to the inner surface of the coated tube with ^{125}I-labeled anti-PSA antibody and represented by B. The B/T ratio was computed for each standard and unknown sample as follows:

$$B/T\ \% = \frac{\text{Standard or sample means counts C.P.M}}{\text{Total radioactivity mean counts C.P.M}} \times 100$$

A standard curve was drawn by plotting the percent value for each standard against the corresponding PSA standard concentration. Total-PSA concentrations of the unknowns were calculated from the standard curve using the mean of their duplicate counts.

Determination of total PSA level in tissues of patients with breast tumors

The IRMA was capable to measure PSA levels in serum and in order to be applicable for determination of PSA in tissue, a standard addition method was performed to ensure that there is no interference if tissue was in use and the IRMA was capable to determine PSA levels in tissue with high accuracy.

$$\text{Recovery \%} = \frac{\text{Actual Value}}{\text{Expected Value}} \times 100$$

The PSA concentration in (ng/ml) of the breast tumor tissue homogenate was estimated according to assay protocol that described as mentioned in the total PSA IRMA kit from Immunotech- Czech Republic, and the ng/ml units were then converted to mg per gram protein.

IRMA determination

The concentration of t-PSA in breast tumor tissue homogenate was determined by the same immunoradiometric assay used for serum t-PSA determination that is mentioned in the total PSA IRMA kit from Immunotech- Czech Republic.

Recovery determination

The assay protocol for the standard addition method for determination PSA was described as mentioned in the recovery determination by total PSA IRMA kit from Immunotech- Czech Republic.

Calculations

The bound (c.p.m) of the reaction mixture (standard PSA was added to tissue homogenate) with ^{125}I -anti PSA antibody, represent the actual value, and the expected value represent the bound (c.p.m.) of PSA in tissue homogenate with ^{125}I –anti PSA antibody plus the bound (c.p.m) of standard PSA with ^{125}I -anti PSA antibody. The recovery % (yield%) was calculated as follows:

Results and Discussion

Determination of t-PSA in sera of breast tumors

Preoperative serum total PSA concentration were measured with an immunoradiometric assay (IRMA) for patients with benign breast tumors (group I), patients with pre- and postmenopausal malignant breast tumors (group II and group III) respectively.The three groups were matched with a group of control subjects (group IV).

Table (1) summarizes the groups, ages and the mean ± SD concentrations of PSA for all the groups, which were determined by using the standard curve in figure (1).

Table (1): Sera t-PSA levels (ng.ml^{-1}) in patients with benighn and malignant breast tumors. (All other detailes are explained in the text).

Group	Patients	Number	Age (year)	PSA ng/ml	P values
I	Bengin breast tumor	29	46.9± 8.4	1.17±0.67	< 0.0001
II	Premenopausal breast tumor	21	35.3± 4.4	0.59± 0.31	< 0.05
III	Postmenopausal breast tumor	27	54.8±5.3	0.5±0.23	< 0.05
IV	Control	32	45.8±8.1	0.37±0.23	

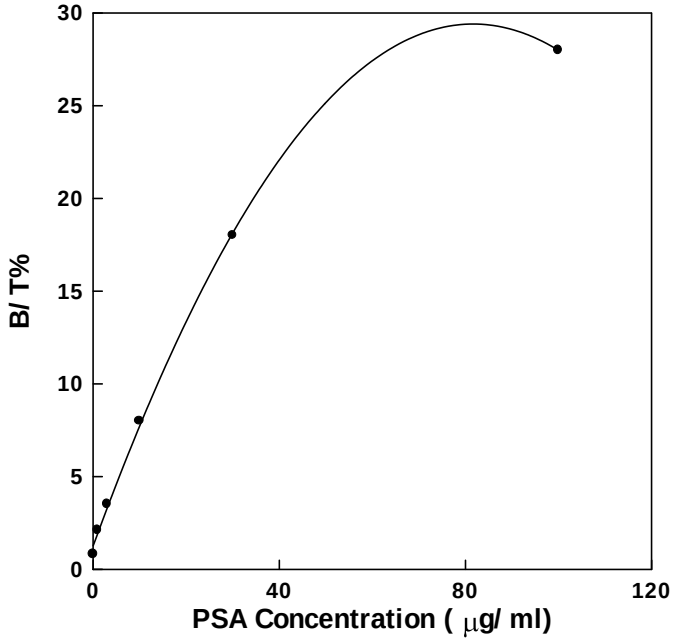

Figure (1): Standard curve of PSA determination in human sera by IRMA method. (All other details are explained in the text).

The mean serum level of t-PSA of the control was 0.37 ng/ml; it's nearly similar to cut-off value 0.34ng/ml [8] and less than the cut-off value 0.015ng/ml [5] and 0.0035 ng/ml [9]. This discrimination may be due to the difference in the sensitivities of PSA assays.

The mean serum level of t-PSA in patients with the three groups (groupI, groupII and groupIII) was found to be (1.17, 0.59 and 0.5) ng/ml respectively. Student's t-test analysis with groupI was very significantly elevated (P< 0.0001) whereas the means serum level of t-PSA in patients with groupII and groupIII were significantly elevated (P< 0.05).

These results indicated that there was an increase in t-PSA levels in the serum of all patients in comparison to the control group. It was speculated that the increase of t-PSA in the serum of patients with groupI and the slight increase of t-PSA in the serum of patients with groupII and groupIII was the result of disrupted hormonal balance in these patients, triggering the aberrant expression of hormone-dependent genes such as PSA [184]. PSA levels in the serum of some women with fibroadenomas or with

breast cyst can attain the same levels as seen in men with prostate cancer, reaching 55 ng/ml in one [10]

These results are nearly similar to those obtained previously by (Black *et al*) [3] serum t-PSA levels in women with breast cancer were significantly higher than healthy women, and in women with breast cysts were significantly higher than women with breast cancer. But another study has shown that serum t-PSA concentrations were significantly lower in women with breast cancer than women with benign breast disease and no significant difference was found between cancer patients and normal women [5]. In another study, no significant difference in serum t-PSA levels was found between breast cancer patients and women with benign breast disease or healthy women [6].

These results indicate that the presence of t-PSA in the serum of patients with breast tumors may be associated with less aggressive tumors [9], these finding support data from other investigators indicated that a high level of PSA in the serum of patients with the breast tumors may be used as a favorable prognostic indicator [11], whereas another investigator went further to represent PSA as a marker with numerous potential clinical application a diagnostic and prognostic tool in breast disease [12].

Determination of t-PSA in breast tumor tissues

Total PSA were measured in breast tumors tissues by immunoradiometric assay (IRMA) for patients with benign breast tumors (groupI), Patients with pre- and postmenopausal malignant breast tumors (groupII and groupIII) respectively.

Table (2) summarizes the groups, ages and the mean ± SD concentrations of PSA for all the groups.

Table (2): Total PSA level and recovery test in tissue of patients with benign and malignant breast tumors. (All other details are explained in the text).

Group	Number	Age (year)	Standard PSA ng/ml	Sample PSA ng/ml	Expected PSA	Actual PSA	Recovery %
I	9	46.5±8.2	1	2.95±1.08	3.95	3.87	98
			2.5		5.45	5.55	101
			7.5		10.45	10.50	100
			20		22.95	23.41	102
II	10	34.8±5.4	1	1.78±0.49	2.78	2.92	105
			2.5		4.28	4.08	96
			7.5		9.28	9.43	102
			20		21.78	22.11	102
III	12	55.6±5.1	1	1.54±0.09	2.54	2.50	98
			2.5		4.04	4.15	103
			7.5		9.04	9.20	102
			20		21.54	21.60	100

The mean concentrations of t-PSA in the tissue patients with the three groups (groupI, groupII and groupIII) were found to be (2.95, 1.78 and 1.54) ng/ml respectively or found to be (3.9, 1.9 and 0.8) ng/mg protein respectively. Student's t-test analysis revealed that the mean concentration of t-PSA in patients in groupI was significantly higher than in groupII and groupIII ($P< 0.001$) whereas the mean concentration of t-PSA in patients with groupII and groupIII were significantly not different ($P< 0.1$).

These results indicate that there was a decrease in t-PSA levels in malignant breast tumors tissues compared with that of benign breast tumors tissues, this finding might be attributed to that in breast cancer, the regulation of PSA was disturbed and the expression may be reduced or lost as the cells lose differentiation. Highest expression of PSA was seen in tissue extracts from patients with benign breast disease [4]. The presence of PSA in these female tissues seems to be associated closely with steroid hormone regulation, especially androgens, progestins, glucocorticoids and meneralocorticoids through their nuclear receptors, but estrogens do not regulate expression of PSA gene directly, but they suppress the up-regulation of the gene by androgens[13-18]. It was also shown that PSA might act as a negative growth regulator in hormone-dependent breast cancer cell lines [19]. The idea of PSA to be a means of defense or protection against cancer was first produced by Eleftherios, *et al* [20], who mentioned that PSA should be considered as a cancer fighter at a tissue level and as a valuable messenger (indicator) at the level of systematic circulation. It has been thus suggested that efforts to produce cancer vaccines or other therapies targeting PSA expression may be the wrong strategy and that treatment approaches to treat prostate and possibly breast, cancer should be directed toward over expression of PSA at the tissue levels [21, 22].

These results are nearly similar to this obtained previously by (Sauter, *et al*.) [23].

These results indicated that the level of PSA in breast tumors tissues inversely correlates with abnormal breast cytology. These finding support data from other investigators indicating that a high level of PSA in the breast may be used as a new biochemical marker for breast tumor prognosis [12, 24-28]. In contrast, other studies have suggested a lack of value of PSA immunoreactivity in breast cancer patients as a general prognostic marker for breast cancer [298-32].

Many investigators detected PSA level in breast tumor tissues and obtained different value >0.05 µg/L [25], 0.31 ng/mg protein [31], > or = 0.03 ng/mg protein [4], ≥ 0.015 ng/mg protein [33], > 4300 ng/L [34], 19.52 µg/ml [35]. This discrimination may be due to the difference in the sensitivities of PSA assays.

These results indicated that there was an increase in t-PSA levels in breast tumor tissues in comparison to the levels in the serum. It was speculated that the source of circulating PSA in females mammary ductal system, as PSA is expressed predominantly in breast tissue and enters its secretions. The concentration of PSA in female sera is approximately 1000 fold lower than that of the breast tissue [12].

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