Screening for Prostate Specific Antigen in Various Types of Breast Tumor

1Labelled Compounds Department, Hot Laboratories Center, Atomic Energy Authority and 2Biochemistry Department, Faculty of Science, Ain Shams University.

The objective of the present study is to investigate a comparative estimation screening method of prostate specific antigen levels in serum samples with immunohistochemistry results for analysis expression of prostate specific antigen in female breast tissues at benign breast hyperplasia and breast cancer. This study was applied on 65 women suffering from breast cancer, 31 of them have benign breast hyperplasia and 34 have malignant breast cancer. Each group was divided into 2 subgroups, pre and postmenopause, both subgroups were compared with 15 normal cases to assess serum and tissue PSA in benign and malignant patients in pre and post menopause state by immunoradiometric assay in blood and immunohistochemistry in tissue. No significant difference in serum total PSA concentration between any of the studied cases was observed. However, there were different variations in the distribution of PSA in the breast tumor tissue, with complete absence of PSA in the tissue samples of the malignant in postmenopausal patients. In conclusion, PSA presence in benign breast hyperplasia in either serum or tissue may show a clinical value regarding the diagnosis and prognosis of the disease.

Keywords: Prostate Specific Antigen, Breast Tumor, CA 15-3, Immunohistochemistry.

Introduction

Proteins that are expressed by both malignant and healthy fetal tissues are recognized as oncofetal. These antigens are associated with cell proliferation and differentiation and are produced in high concentrations in pregnancy and malignancy. Their biological role in malignancy is the suppression of the host’s immune system, while in pregnancy they affect the maternal immune response, generating maternal tolerance toward the embryo. The levels of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), squamous cell carcinoma antigen (SCC), cancer antigen 15-3 (CA 15-3), mucin-like carcinoma-associated antigen (MCA), tissue polypeptide-specific antigen (TPS), carbohydrate antigen 19-9 (CA 19-9), and prostate-specific antigen (PSA) in maternal serum (MS), umbilical cord serum (UC), and amniotic fluid (AF) have different roles in the assessment of pregnancy and malignancy. All these antigens, except CA 15-3, are oncofetal. The presence of considerable concentrations of AFP, hCG, CEA, CA125, SCC, MCA, TPS, CA 19-9, and PSA in AF during pregnancy may be attributed to their involvement in biological functions associated with fetal development, differentiation, and maturation. MS CEA, CA 15-3, and CA 19-9, in contrast to all the others, are not influenced significantly by pregnancy and thus remain reliable tumor markers in monitoring malignancy in pregnant patients.(Yu and Berkel 1999, Sarandakou, Protonotariou et al. 2007).

To date, no true tissue specific antigen has been discovered. Prostate-specific antigen (PSA) was initially reported to be a tissue specific protein. The name prostate-specific antigen has been given to a protein that now is known not to be prostate-specific. PSA detected in the seminal fluid and produced by normal and abnormal epithelial cells of the prostate gland. PSA is a 33 kDa glycoprotein, with serine protease activity, seminal plasma contains about 1 million micrograms/L of PSA and is the richest source of PSA reported, and it is produced by various tissues in the human body. Its expression levels may be elevated during benign
and neoplastic cell growth in the prostate and in a number of other human malignancies. Male serum PSA is usually less than 4 micrograms/L. In nonprostatic tissues, PSA exists mainly in its free molecular form, but PSA-ACT complex is also present in most of the fluids that contain PSA, such as breast secretions and amniotic fluid. The detection of PSA is also useful in monitoring the efficacy of anticancer treatment in malignant prostatic adenocarcinoma, (Borchert, Melegos et al. 1997, Diamandis and Yu 1997).

Prostate-specific antigen (PSA) was recently found in 30% of female breast tumors. PSA has been shown to be expressed in many forms of female tissues. The breast is a major female organ that is able to produce PSA (Yu and Berkel 1999, Kamenov, Todorova et al. 2001). PSA is detected in both normal and abnormal breast tissues, as well as in various breast fluids including milk, nipple aspirate, and cyst fluid. The biologic fluid with the second highest PSA concentration, however, is nipple aspirate fluid from the female breast (up to about 5000 micrograms/L), and the third is milk from lactating women (up to 300 micrograms/L). Androgens and progesterone, via their receptors, regulate the production of PSA in breast tissue. Clinical studies demonstrate that PSA in breast cancer is associated with the expression of estrogen receptor and progesterone receptor.

Women with PSA-positive breast cancer have better disease-free survival as well as overall survival than those with PSA-negative breast cancer. PSA levels in nipple aspirate fluid may be indicative of breast cancer risk. High concentrations of PSA are found in amniotic fluid and male serum, and levels change with gestational age (Mitchell, Sibley et al. 2002, Narita, Cimpean et al. 2006). Pregnant women have elevated serum PSA. PSA levels in serum also vary during menstrual cycles and increase in women with excess androgen. Clinical implications of PSA in amniotic fluid and female serum have been suggested. The immunoexpression of prostate-specific antigen in breast cancers has been well established, but the role of this extra-prostate PSA appears to be a complex, poorly understood and of doubtful prognostic value (Giai, Yu et al. 1995, Mitchell, Sibley et al. 2002, Narita, Cimpean et al. 2006).

The gene expression and protein production of PSA in nonprostatic tissues are under the regulation of steroid hormones via their receptors. Androgens, glucocorticoids, and progestins up-regulate the PSA gene expression, resulting in an increase of protein production. Estrogen by itself seems to have no effect on PSA regulation, but it can impair PSA production induced by androgen. It remains unknown whether PSA is enzymatically active and what is the physiologic role of PSA in nonprostatic tissues. It is speculated that PSA may be involved in the regulation of growth factors (Sauter, Lininger et al., 2004).

A comparative estimation screening method of prostate specific antigen levels in blood serum with immunohistochemistry for analysis of expression of prostate specific antigen in female breast tissue at benign breast hyperplasia and breast cancer is the aim of this study.

**Subjects and methods**

**Subject**

A total of 65 Egyptian women aged 30-69 years were recruited for this study and selected from outpatient’s clinic at the National Cancer Institute. Physical examinations were processed by physicians in the Medical Oncology Unit and routine clinical examination was further demanded for diagnosis. The patients were grouped according to their clinical status into 4 different groups, benign breast hyperplasia premenopause, benign breast hyperplasia postmenopause, breast cancer premenopause and breast cancer postmenopause.

**Diagnosis**

After physical examination, patients were forwarded for further investigations that include complete blood count (CBC), serum Cancer Antigen 15.3 (serum CA 15.3) and prostate specific antigen (PSA), alkaline phosphatase activity, as well as Calcium. Furthermore, immunohistochemistry examination of prostate specific antigen was fulfilled for breast biopsies.

**Blood collection and sampling**

Venous blood samples were generally collected from patients at the outclinic unit. Each blood sample was divided into two vacutainer labeled tubes, with or without anticoagulant (EDTA). Blood was collected in EDTA used in analysis of complete blood count (CBC). In the other tube, blood was allowed to stand for 1 hour at room temperature and then centrifugated at 5,000 rpm for 5 min and serum was aspirated and preserved at -70°C for the analysis of Cancer antigen 15-3 (CA 15-3) and prostate specific antigen (PSA), Alkaline phosphatase and Calcium levels.
**Determination of CA 15.3**

CA15.3 was carried out by The AxSYM CA 15-3 assay which is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative measurement of CA 15-3 values in human serum and plasma (EDTA) (Hayes, Zurawski et al. 1986, Sekine 1987, Shimokata, Totani et al. 1988). The Abbott AxSYM CA 15-3 assay is based on the 115D8 and DF3 antibodies which are available exclusively through Fujirebio, Inc.

**PSA in serum**

PSA was determined using PSA IRMA Kit supplied by IZOTOP Inc. Ltd. The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay system (Ooi and Escares 1991, Abbate, Musci et al. 1996).

**Determination of Ca in serum**

Calcium ions were determined using SPECTRUM Diagnostics Kit. The reaction based on the Ca reaction with o-cresolphthalein complexone (o-CPC) under alkaline conditions to form a violet colored complex. The addition of 8-hydroxyquinoline prevents interference by magnesium and iron. It was determined by measuring the increase in absorbance at 578 nm, (Kessler and Wolfman 1964).

**Determination of Alkaline phosphatase in serum:**

Alkaline Phosphatase activity was determined using SPECTRUM Diagnostics Kit. The reaction was based on the conversion of p-Nitrophenyl phosphate to p-Nitrophenol and phosphate by alkaline phosphatase. The increase of absorption at 405 nm is proportional to the alkaline phosphatase concentration in the sample, (Moss 1982, Moss 1987).

**PSA immunohistochemistry**

Benign & malignant tissue samples were obtained directly at the operating room temperature, kept in ice. Fat & necrotic tissue were removed and the tissue samples were divided into 3 aliquots and stored at -80 C until being used, (Narita, Raica et al. 2005).

**Results**

This study included a total of 80 cases divided into three major groups. Healthy subjects with no history of any medical or genetic disease (n = 15). The second group consisted of female subjects diagnosed with benign breast cancer by the personal of the National Cancer Institute (n = 31), those were further divided into 2 subgroups according to their menopause status as pre- (n = 19) and postmenopause (n = 12). The third group consisted of female subjects diagnosed with comprised malignant breast cancer by the personal of the National Cancer Institute (n = 34), those were further divided into 2 subgroups according to their menopausal status as pre (n = 14) and postmenopause (n = 20), (Table 1).

**TABLE 1. Data of the studied subjects.**

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>Malignant</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-Menopause</td>
<td>Pre-Menopause</td>
<td>Post-Menopause</td>
</tr>
<tr>
<td>Number</td>
<td>12</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>52.50 ± 5.89</td>
<td>30.47 ± 7.36</td>
<td>58.5 ± 7.29</td>
</tr>
<tr>
<td>Range</td>
<td>45 - 63</td>
<td>17 - 41</td>
<td>49 - 75</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD.

Figure 1 shows serum Calcium concentration and Alkaline Phosphatase activity for the studied subjects. Our data showed only a significant difference in serum Calcium concentration between benign breast cancer patients and normal subjects. In addition, the data of the present study did not found any significant difference in serum Alkaline Phosphatase activity between any of the studied subjects using unpaired “t” test.

Regarding serum CA-15.3, the data of the present work showed a significant difference in serum CA 15.3 concentrating when comparing benign and malignant patients with normal subjects. Also, there was a significant difference observed when comparing all the subgroups with normal subjects, (Table 2).
For the serum PSA, the results did not show any significant difference in serum Total PSA concentration between any of the studied subjects. On the other hand, the histopathological immunostaining results showed different variations in the distribution of PSA in the breast tumor tissue, with a complete absence of the PSA in the tissue samples of the malignant in postmenopausal patients, (Table 3).

Figure 2 shows the distribution of the different degrees of positivity of the PSA in the tumor tissues presented as percentage of the total samples studied.

**Discussion:**

Breast cancer is a major concern worldwide and is responsible for one of the highest causes of death. The chance that breast cancer will be responsible for a woman’s death is about 1 in 35 (about 3%). In 2007, about 40 460 women will die from breast cancer in the United States, 1% of women are diagnostic every year with breast cancer and 85% are diagnostic with advanced stages of disease (Narita, Anghel et al., 2008).

Researchers are trying to discover new biomarkers for diagnosis, prognosis, treatment monitoring and to develop new drugs that might work better against breast cancer (Narita, Anghel et al., 2010) which may help greatly in early detection of malignancy (Poh, Jayaram et al., 2008). Different factors such as high body mass index, advanced age, family history of breast cancer, a long menstrual history, use of oral contraceptives, exposure to radiation, no childbearing or giving birth to the first child after age 30 are among possible risk factors for breast cancer (Razavi, Ghajarzadeh et al., 2015). Prognostic and predictive markers for breast cancer are a mixture of host factors (e.g., age, menopausal status and inflammatory response) and tumor features (e.g., tumor size, histological grade, nodal involvement, vascular invasion, hormone receptors, growth factors and their receptors, cell proliferation and angiogenesis markers and various DNA or genetic alteration (Porter-Jordan and Lippman 1994).

**TABLE 2. CA-15.3 concentrations in the studied groups.**

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>Malignant</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-15.3 (U/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post- Menopause</td>
<td>42.84 ± 8.51*</td>
<td>32.87 ± 22.53*</td>
<td>19.13 ± 3.28</td>
</tr>
<tr>
<td>Pre- Menopause</td>
<td>39.02± 6.13*</td>
<td>46.66± 8.83*</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD.

* p value is significant against Normal subjects.

**TABLE 3. Total PSA concentrations in the studied groups.**

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>Malignant</th>
<th>Normal</th>
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<tbody>
<tr>
<td>Total PSA (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post- Menopause</td>
<td>0.036 ± 0.036</td>
<td>0.025 ± 0.009</td>
<td>0.024 ± 0.006</td>
</tr>
<tr>
<td>Pre- Menopause</td>
<td>0.028± 0.015</td>
<td>0.042± 0.044</td>
<td></td>
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</table>

Data are shown as mean ± SD.

Fig. 1. Comparison of Serum Calcium and alkaline phosphatase in the studied subjects.
Fig. 2. Distribution of the different grades of PSA positivity in the pathological tissue sections of the different studied patients.

Fig. 3. Immunohistochemical staining of PSA: A- benign postmenopause group, B-benign premenopause group, C-malignant postmenopause group, D-malignant premenopause group (Brown color indicates the presence of PSA).
Although tumor size, axillary lymph node status, and tumor grade are still among the most important prognostic factors (Mohammadizadeh, Ranaee et al., 2012), physicians have difficulty identifying patients who need adjuvant treatment and who will benefit from the treatment because the available markers are not appreciably sensitive and specific. The ultimate goal of using prognostic and predictive markers is to allow physicians to accurately differentiate patients who need postsurgical treatment and to appropriately tailor therapy to their specific needs (Giai, Yu et al. 1995) and decide whether and how to treat breast cancer patients after a local surgery with asignificant impact on survival of patients and health care cost (Elledge, McGuire et al., 1992).

This study aimed to investigate a comparative estimation screening method of prostate specific antigen levels in blood serum with immunohistochemistry results for analysis expression of prostate specific antigen in female breast tissue at benign breast hyperplasia and breast cancer.

CA15-3 was a high-molecular-mass mucin-like glycoprotein expressed at the luminal surface of most secretory epithelia and associated with mammary tumors. Clinical uses of this marker include monitoring of patients with breast cancer, prognosis, recurrence and metastasis (Atoum, Nimer et al., 2012). CA15-3 level correlates exclusively with tumor size; higher CA15-3 serum level was found in advanced cancer stages and higher grades and metastasis (Theriault, Hortobagyi et al., 1989). CA15-3 level increased in 10% stage I breast cancer disease, 20% stage II disease, 40% stage III disease, and 75% with stage IV disease (Duffy 2006). Elevated levels of this biomarker were detected among certain benign diseases, primary breast carcinoma (Coveney, Geraghty et al., 1995) and in patients with advanced adenocarcinomas (Nicolini and Carpi 2000).

Aging and menopause disturb the hormonal statusamong females and increase the chance of breast cancer development (Pike, Pearce et al., 2004), at the same time, serum CA15-3 level showed a significant increase within elderly menopause patients with breast cancer (Dehaghami, Ghiam et al., 2007).

In this study, it was found that there was a significant difference in serum CA 15.3 concentrationon comparing benign and malignant patients with normal subjects. In addition, except for the malignant premenopause group, there was a significant difference on comparing all the subgroups with normal subjects. The previous findings agree with those of Gourevitch(Gourevitch, von Mensdorff-Pouilly et al., 1995).

Although the data obtained disagree with the data of Kumpulainen, Keskikuru et al., 2002 who assessed CA 15.3 pre or post-operation patients with primary breast cancer, and compared it as a prognostic factor to the conventional prognostic factors to evaluate the usefulness of tumor marker CA 15.3. Kumpulainen, Keskikuru et al., 2002 found that this tumor marker, that is determined at the time of primary diagnosis as a prognostic factor in breast cancer, is correlated with more advanced stage, distant metastases, advanced nodal involvement, higher grade, and lower hormone receptor content.

The results obtained showed that there is no significant difference of serum total PSA concentrations between any of the studied subjects, but there is a slight increase in serum PSA of younger female patients with premenopausal benign hyperplasia. This result agrees with that of Giai et al. who found that there is no substantial difference between serum PSA levels from normal women and women with breast cancer.

On the contrary, the results disagree with those of Rasavi et al. who found that total and free PSA levels are significantly higher in women with malignant breast masses compared with women who had benign breast masses.

Conclusion

With PSA distributed in pre and postmenopausal females with benign and malignant breast tissue, it was found that PSA is absent only in postmenopausal females with malignant breast tissue.

References


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فحص دالة أورام البروستاتا في مختلف أنواع أورام الثدى

أحمد سامي البيومي \۱- قسم الكيمياء الحيوية- كلية العلوم- جامعة عين شمس- مصر
FAWZY M, ABDEL-GHAFFAR et al. \۲-قسم المركبات المرقمة- شعبة انتاج النظائر والمصادر المشعة- مركز المعامل الحارة- هيئة الطاقة الذرية

الهدف من هذه الدراسة هو الفحص والتشخيص المبكر لداء أورام البروستاتا في مصل الدم وكذلك أنسجة الثدى

في الأداب المصاب بداء أورام الثدى المديدة وغير المديدة. أجريت هذه الدراسة على 65 امرأة مصابه بأورام الثدى، 34 أسافين بأورام حميدة والمجموعة الثانية 31 أورام سرطانية. كل مجموعة تم تقسيمها إلى مجموعتين فرعيتين على أساس قبل انقطاع الطمث وعند انقطاع الثدى. فحص تواجد دالة أورام البروستاتا في مصل الدم. وافق الفحص المبكر لداء أورام البروستاتا في الأداب السرطانية في حالة معنوية غير معروفة في حالات أورام البروستاتا والثدي السرطانية في حالة معنوية غير معروفة. ومتاحا وجد حالة أورام البروستاتا في ثدى السرطانية سواء في مصل الدم أو الأنسجة تظهر قيمة بشأن تشخيص سريري وتشخيص المرض.