Prostate-specific Membrane Antigen Levels in Sera from Healthy Men and Patients with Benign Prostate Hyperplasia or Prostate Cancer


ABSTRACT

Prostate-specific membrane antigen (PSMA) serum levels have been proposed to be of prognostic significance in patients with advanced prostate disease. The objective of the present study was to confirm PSMA serum expression by Western blot techniques, to determine whether such data could assist in the differentiation of benign from malignant prostatic disease, and to determine the suitability of serum PSMA measurements in predicting recurrent or progressive prostate malignancies. We measured PSMA, a transmembrane glycoprotein identified in prostate epithelial cells, in the sera of 236 normal individuals and cancer patients by Western blot analysis. Within the normal male population, PSMA levels increase with age and were found to be significantly elevated in subjects more than 50 years of age when compared to those of younger men. We did not confirm previous reports that serum PSMA measurements could distinguish late-stage prostate carcinoma from early-stage prostate carcinoma, nor did we find PSMA to be more effective than prostate-specific antigen in monitoring prostate cancer patient prognosis. Furthermore, we found elevated serum PSMA in healthy females, and, similar to the healthy male population, the levels increased with age, with the highest levels found in the sera from breast cancer patients. These latter observations further support that PSMA is not a specific biomarker for prostate cancer and that a variety of normal and diseased tissue may contribute to the serum levels of PSMA.

INTRODUCTION

PSA\(^3\) is considered the best tumor marker for CaP; however there are drawbacks to its use. For example, PSA cannot always accurately differentiate BPH from CaP, especially when the PSA levels are between 2 and 20 ng/ml (1–3). Some improvement in differentiating BPH from CaP has been achieved when both the free and total PSA isoforms are measured (1–7). Although using free:total PSA or percentage of free PSA improves test specificity, 5–10% of carcinoma cases may be missed (6, 7). Other shortcomings may include the inability to distinguish clinically important CaPs from indolent carcinomas and the inability to predict metastatic potential and therefore patient prognosis. Although the clinical utility of PSA for diagnosis and treatment monitoring of CaP is not disputed, other biomarkers need to be identified to fill in the diagnostic/prognostic gaps.

A transmembrane glycoprotein identified in prostate epithelial cells, designated PSMA, is a novel biomarker that may have diagnostic and therapeutic potential (8). This\(\text{M} \_100,000\) protein was found to be highly expressed in both primary and metastatic prostate carcinomas and was up-regulated in many patients receiving hormone ablation therapy (9, 10). An isotope-conjugated form of the 7E11-C5.3 antibody that binds to an internal epitope of PSMA, designated CYT-356, has been successfully used to image prostate carcinoma lesions in vivo (11). Investigators (12, 13) also have reported the detection of PSMA in serum from CaP patients by Western blot analysis and noted that PSMA was of prognostic significance, especially in patients with metastatic disease, suggesting a correlation with poor prognosis (14).

The overall purpose of the present study was to determine what, if any, potential clinical utility serum PSMA may have in the diagnosis and monitoring of patients with CaP. First, we wished to confirm the reliable and reproducible measurement of serum PSMA by Western blot analysis. More specifically, we examined whether PSMA serum levels, as determined by Western blot analysis, could assist in the differentiation of BPH from CaP and could be more reliable than PSA in detecting recurrent or progressive disease.

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\(^3\)The abbreviations used are: PSA, prostate-specific antigen; BPH, benign prostate hyperplasia; CaP, prostate cancer; PSMA, prostate-specific membrane antigen; CV, coefficient of variation.

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MATERIALS AND METHODS

Patients and Serum Samples. All serum samples were obtained from the serum bank maintained at Eastern Virginia Medical School. Sera from normal males (as determined by negative digital rectal examination (DRE) and PSA values < 4.0 ng/ml) and normal females (i.e., females with no evidence of breast cancer at the time of specimen collection), ages 22–73 years, constituted the control populations. The age range for normal males < 50 years of age was 25–39 years, with a mean age of 34 years, whereas for normal males > 50 years of age, the age range was 51–73 years, with a mean age of 60 years. The BPH group consisted of patients with bladder outlet obstructive symptoms and elevated PSA values determined by negative digital rectal examination (DRE) and PSA (4.0 ng/ml) and normal females (i.e., females with no evidence of breast cancer at the time of specimen collection), ages 22–73 years, constituted the control populations. The age range for normal males < 50 years of age was 25–39 years, with a mean age of 34 years, whereas for normal males > 50 years of age, the age range was 51–73 years, with a mean age of 60 years. The BPH group consisted of patients with bladder outlet obstructive symptoms and elevated PSA (4.0 ng/ml) who had undergone transrectal ultrasound biopsy that revealed BPH. These patients had not or were not receiving treatment at the time of serum collection. Prostatitis included a group of patients with a chronic symptom complex attributed to a prostatic source, based on the absence of evidence of other prostatic pathology, i.e., obstruction, infection by urine culture, abscess, or carcinoma. Sera from females documented as having breast carcinoma were all post-treatment specimens and included patients with early-stage (T1 and TII) and late-stage (TIII and TIV) disease, with and without lymph node and/or bone metastases. Male serum samples were representative of all clinical stages of prostatic disease (T1, T2, T3, N+M+) and included pre- and post-treatment specimens from different individuals. Pools of various sera representing both high and low PSMA values were generated and then titrated under conditions of the Western blot analysis to establish a minimum protein concentration that would ensure the detection of PSMA, if present, in all samples. Protein concentrations were determined using Pierce’s BCA protein assay (Rockford, IL) according to manufacturer’s directions.

Electrophoresis and Western Blotting. Serum samples for Western blot analysis (100 μg total protein/lane) were electrophoresed under reducing conditions on a 7.5% SDS-PAGE minigel. The separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA) for 40 min at 80 V. The membranes were blocked overnight or for 2 h at room temperature in Tris-buffered saline (pH 7.5) containing 0.2% I-block (Tropix, Bedford, MA), 1% milk, and 0.1% Tween-20 (TBS-T). The membranes were subsequently incubated with 20 μg/ml 7E11-C5 antibody for 2 h, followed by three washes in TBS-T and a 1-h incubation with goat antimouse horseradish peroxidase-labeled secondary antibody at 0.25 μg/ml in blocking buffer (TBS-T). The membranes were again washed in TBS-T and Tris-buffered saline and developed in Amersham’s chemiluminescent reagent (enhanced chemiluminescence, Arlington Heights, IL) according to manufacturer’s instructions. Blots were visualized by exposing the enhanced chemiluminescence-reacted blot to X-ray film. Images were scanned and quantified using the Whole Band Analyzer system from Bio Image (Ann Arbor, MI). A standard curve using LNCaP lysate (15) was generated by loading known quantities of lysate as determined by the BCA protein assay. Densitometric evaluation was based on integrated intensity values because this value, unlike simple intensity, takes into account the volume of the band in addition to the band intensity. Therefore, PSMA is expressed in densitometer (integrated intensity) units. Verification of the specificity of the detected band was performed by incubating the 7E11-C5 antibody with an excess of the 19-mer PSMA peptide (MWNLL-HEHTDASVATARRPR; Ref. 15) before the addition of antibody to the membrane. All samples giving a negative PSMA value on Western blot were repeated three times to ensure the validity of the negative results.

Statistical Analysis. Comparisons between the mean PSMA values were made by using the Student’s t test and the Mann-Whitney or Kruskal-Wallis tests. For all analyses, P < 0.05 was considered statistically significant. The normal PSMA range was defined as the mean ± 3 SDs from the mean.

RESULTS

We have been able to confirm the presence of PSMA in serum by Western blot analysis in both males and females and to verify its identity by blocking experiments using the PSMA 19-mer peptide (Fig. 1). Serum samples negative for PSMA remained so after multiple analyses of the sample. The interassay variation for normal males less than and more than age 50 years was monitored. Males < 50 years of age showed a mean value of 0.940 and a CV of 27%, whereas normal males > age 50 years showed a mean value of 0.9967 and a CV of 16%. Intra-assay variability for these two groups indicated a CV of 8.8%.

A total of 236 individual serum samples were evaluated for the presence of PSMA. A summary of the patient demographics, PSMA range and mean serum levels, and statistical results is shown in Tables 1 and 2. Comparison of the mean PSMA levels in normal males versus the mean PSMA levels in patients with benign prostate disease (both BPH and prostatitis) and CaP with pathological stage T1/T2 or T3 cancer is shown in Fig. 2A. For normal males < age 50 years, PSMA ranged from 0 (no detectable PSMA) to 9.564, with a mean value of 1.56, whereas the range and mean serum levels for
Table 1  Summary of patient demographics, PSMA range, mean serum levels, and statistical data in healthy male population and patients with benign (BPH or prostatitis) or malignant prostate disease

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>No. of patients</th>
<th>Mean age (yr)</th>
<th>Age range (yr)</th>
<th>Mean PSMA (median)</th>
<th>PSMA range</th>
<th>SE</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal male &lt; 50 yr</td>
<td></td>
<td>22</td>
<td>34</td>
<td>25–39</td>
<td>1.56 (0)</td>
<td>0–9.56</td>
<td>0.53</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal male &gt; 50 yr</td>
<td></td>
<td>25</td>
<td>60</td>
<td>51–73</td>
<td>3.48 (2.49)</td>
<td>0–16.8</td>
<td>0.79</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td></td>
<td>25</td>
<td>67</td>
<td>54–81</td>
<td>1.75 (1.08)</td>
<td>0–10.8</td>
<td>0.48</td>
<td>0.78</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Prostatitis</td>
<td></td>
<td>25</td>
<td>66</td>
<td>46–89</td>
<td>2.64 (1.14)</td>
<td>0–16.8</td>
<td>0.8</td>
<td>0.28</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>CaP T1/T2</td>
<td></td>
<td>25</td>
<td>66</td>
<td>55–76</td>
<td>1.7 (0.27)</td>
<td>0–17.3</td>
<td>0.73</td>
<td>0.89</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>CaP T3</td>
<td></td>
<td>26</td>
<td>67</td>
<td>46–93</td>
<td>6.82 (3.31)</td>
<td>0.25–31.5</td>
<td>1.75</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>CaP T2/T3 pretreatment</td>
<td></td>
<td>28</td>
<td>65</td>
<td>51–87</td>
<td>3.09 (1.95)</td>
<td>0–19.4</td>
<td>0.79</td>
<td>&lt;0.02</td>
<td>&gt;0.5</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>CaP T2/T3 (PSA &lt; 4), posttreatment</td>
<td></td>
<td>26</td>
<td>67</td>
<td>51–79</td>
<td>6.06 (4.26)</td>
<td>0–18.36</td>
<td>1.14</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td></td>
<td>9</td>
<td>67</td>
<td>58–79</td>
<td>6.64</td>
<td>2.14–18.36</td>
<td>1.8</td>
<td>0.006</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Radical</td>
<td></td>
<td>16</td>
<td>62</td>
<td>51–73</td>
<td>5.73</td>
<td>0–17.55</td>
<td>1.5</td>
<td>0.006</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Probability when compared to normal male < 50 years.
<sup>b</sup> Probability when compared to normal male > 50 years.
<sup>c</sup> Probability when compared to posttreatment.

Table 2  Summary of patient demographics, PSMA range, mean serum levels, and statistical data in healthy female population and cancer patients

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of patients</th>
<th>Mean age (yr)</th>
<th>Age range (yr)</th>
<th>PSMA range</th>
<th>Mean PSMA (median)</th>
<th>SE</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal female &lt; 40 yrs</td>
<td>13</td>
<td>27.8</td>
<td>22–37</td>
<td>0–4.2</td>
<td>0.72 (0.16)</td>
<td>0.364</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal female &gt; 40 yrs</td>
<td>12</td>
<td>48.9</td>
<td>40–64</td>
<td>0.32–5.6</td>
<td>1.59 (0.77)</td>
<td>0.55</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Breast cancer patients</td>
<td>10</td>
<td>68.9</td>
<td>45–91</td>
<td>0.92–13.9</td>
<td>6.33 (5.46)</td>
<td>1.54</td>
<td>&lt;0.001</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

<sup>a</sup> Probability when compared to normal female < 40 years.
<sup>b</sup> Probability when compared to normal female > 40 years.

normal males > age 50 years were 0–16.834 and 3.48, respectively. The difference in mean PSMA levels between the two normal populations was statistically significant (P < 0.01). Individuals with BPH, prostatitis, and stage T1/T2 CaP showed PSMA levels varying from 0–17.266, with a mean value of 1.75, 2.64, and 1.7, respectively. None of these values was found to be significantly different from those of the normal males <50 years of age (Table 1), and only CaP T1/T2 serum PSMA levels were significantly different from the normal male group > 50 years of age (P < 0.05). Serum PSMA varied from 0.25–31.5 in the stage T3 CaP group, with a mean of 6.82. This value was significantly different from that of the normal males < 50 years (P < 0.01) but was not different from normal males > 50 (P = 0.09). In addition, comparison of PSMA values for BPH and CaP patients showed that BPH could be distinguished from patients with late-stage but not early-stage cancers (P = 0.007 for T3 and P = 0.95 for T1/T2, respectively).

Mean PSMA serum levels in normal females and patients with breast carcinoma are shown in Fig. 2B. Similar to the normal male population, PSMA levels are significantly different (P = 0.015) in older (>40 years) versus younger (<40 years) individuals, with mean values of 0.72 and 1.59, respectively. Breast cancer patients had a significantly higher serum PSMA level than both normal female groups (P < 0.001 and P = 0.0025 compared to normal females <40 years and normal females ≥ 40 years, respectively).

Evaluation of PSMA expression in the normal male population by age and race is shown in Fig. 2, C and D, respectively. The mean values rise with increasing age (Fig. 2C), although only the comparison of individuals < 35 years with those > 55 years showed a statistically significant difference in the mean PSMA (P = 0.02). When age and race are considered (Fig. 2D), mean PSMA levels appear elevated in the African-American population, with African-American males < 55 years having PSMA levels almost equivalent to those of non-African-American males > 55 years. However, the difference in mean PSMA between the non-African-Americans < 55 years and the respective group of African-Americans was not statistically significant (0.05 < P < 0.1). In contrast, the former differed significantly from African-Americans > 55 years (P = 0.01). Within the African-American population, no significant difference was observed between the PSMA values of younger (<55 years) versus older (>55 years) individuals (P > 0.2).

Fig. 3 shows the mean PSMA serum levels in single point pretreatment and posttreatment specimens from separate groups of CaP patients (age range, 51–87 years) having PSA values < 4 ng/ml. PSMA serum levels were found to vary widely, with PSMA levels significantly different from the normal male population < 50 years (pretreatment, P < 0.02; posttreatment, P < 0.001), but not from the normal males >50 years (Table 1). In the posttreatment group, this difference was not dependent on the type of treatment received (Table 1; Fig. 3B). PSMA serum levels in the posttreatment group were significantly different from those of the pretreatment group (P < 0.02).

Representative examples of pretreatment and posttreatment serial PSMA and PSA serum levels in patients who underwent radical prostatectomy or received either hormone or X-ray ther-
apy are shown in Fig. 4. In most cases, PSMA and PSA levels paralleled one another (Fig. 4, A, B, and D), or PSMA showed a transient increase (Fig. 4, C and E) before falling to lower levels.

**DISCUSSION**

We have been able to verify the presence of PSMA in serum using Western blot analysis, and we have used this technique to determine the levels of PSMA in sera from male and female subjects. Within the normal groups, we found an increase of PSMA with age. In a study conducted by Oesterling et al. (16), as well as in data obtained on normal PSA values from nearly 4000 individuals in our Eastern Virginia Medical School database (17), PSA has consistently indicated an age (as well as race) difference in normal serum values. It is not unreasonable to conjecture that this may also hold true for PSMA expression. Furthermore, similar to PSA, there also appear to be differences in the levels of PSMA expression in the normal male population based on race. Although our study demonstrates higher levels of PSMA in African-Americans, probably due to the small sample size, this difference was not found to be statistically significant.
Determining the correct normal age group for comparison with the cancer population is of paramount importance. Therefore, to determine the significance of PSMA measurements in the cancer population, an age-matched (>50 years) healthy male population must be the yardstick for comparison. As an example, when using the PSMA values of the healthy male population 50 years of age, late-stage cancers (T3) could be distinguished from the normal male population. By contrast, when the comparison was made using the age-matched (>50 years) healthy male population, there was no significant difference in PSMA levels between this normal group and T3 cancer patients. However, this age-matched normal population did have a significantly greater level of serum PSMA than did T1/T2 patients, i.e., normal males older than 50 years had higher levels of PSMA than did T1/T2 cancer patients.

Examination of single point pretreatment and posttreatment sera from patients with PSA levels within the normal range showed a large proportion of patients in both groups with elevated serum PSMA as compared to the mean PSMA values for normal males < 50 years of age. Although PSA levels in these samples were at or below the norm (4 ng/ml), approximately 57% of the pretreatment group and 86% of the posttreatment group had PSMA levels above the mean PSMA level for the <50 years normal group. When compared to the mean serum level for the >50 years group, 32% and 62% of the pretreatment and posttreatment patients, respectively, had elevated PSMA levels. In such cases, PSMA evaluation may indicate prostate pathology that cannot be assessed by PSA alone.

Evaluation of PSA and PSMA levels in serial samples for individuals receiving various treatment strategies in our study indicated that in all instances, PSMA measurements did not lie outside the normal range established for an age-matched (>50 years) healthy male population at any point in the treatment cycle. In some specimens (patient 3), PSMA levels fell as PSA levels rose. Additionally, none of the individuals monitored in this study showed PSMA values outside the normal range for a tumor-free population < 50 years. These findings are in contrast to data presented by other investigators indicating that from 1 year to >4 years postoperatively, PSMA values were well above the normal range and correlated with poor prognosis (13, 14). These conclusions may have resulted from setting the cutoff too low, based on the younger age of the control population.

The presence of PSMA in non-prostate normal and malignant tissues, including the breast and small intestine, has been confirmed by many investigators using reverse transcription-PCR, although the levels of mRNA in these tissues are approximately 5-fold lower than those in prostate tissues (18). PSMA, particularly its expression in the vascular endothelium of a wide variety of tumor tissues (19), has also been associated with angiogenesis. Reverse transcription-PCR data from our laboratory further show expression of PSMA in benign and malignant uterus and ovarian tissue (data not shown). A more complete evaluation of PSMA in the female population will be presented in a subsequent report. However, these phenomena raise the question as to the prostate tissue specificity of PSMA and the possible extraprostatic source of serum PSMA. Is serum PSMA actually a reflection of a diseased prostate, or are there other conditions, including contributions from normal tissues like the small intestine and advancing age, that contribute to serum PSMA levels in all individuals?

The results obtained in our study support previous reports that Western blotting procedures can detect PSMA antigen in serum (12). However, our results do not confirm previous investigations (13, 14) that suggest serum PSMA measurements can be used to distinguish late-stage from early-stage CaP or monitor regressors more effectively than PSA. Perhaps with the generation of new antibodies to the external domain of the
PSMA molecule (19, 20), more sensitive serum PSMA tests and measurements will be possible in the future.

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