Possible factors influencing high serum Prostate-Specific Antigen (PSA) in Indonesian patients with Benign Prostatic Hyperplasia (BPH)

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Abstract

Benign prostatic hyperplasia (BPH) cases in Indonesia frequently associated with high serum prostate specific antigen (PSA). To explore possible factors that could increase serum PSA level, we performed a retrospective, cross-sectional study on 805 consecutive patients in Sumber Waras and Dr. Cipto Mangunkusumo Hospitals from 1994 to 1997. Clinical manifestations were evaluated and prostate biopsies were performed if indicated. Complete histopathological data were only available in 82 BPH patients with no urinary retention from 1998-1999 and a thin section of paraffin blocks of BPH patients which still could be found from 1994-1999 was analyzed using flow cytometer to obtain the S-phase fraction as a parameter of proliferative activity. From 805 patients, 461 (57%) presented with urinary retention and need to be catheterized. Catheterization significantly increased PSA level if compared to non-catheterized patients (16.3 vs. 6.8 ng/mL, p=0.000). Another data of 82 uncatheterized patients from 1998-1999 has revealed that 79 patients (96.3%) had chronic prostatitis and 19 (23.2%) showed the presence of prostatic-intraepithelial neoplasia (PIN) with an increase of PSA level (5.4 ng/mL). The S-phase fraction of BPH without PIN cases was significantly higher in cases with PSA > 4 ng/ml than patients with PSA ≤ 4 ng/ml (13.1% vs. 8.9%, p=0.008). As conclusion, the high serum PSA level was mostly due to urethral catheterization and increased prostate volume. There was a tendency of increasing PSA in subclinical inflammation and PIN. Cases with high PSA also showed high proliferative activities which is suggestive of mitogenic activity. (Med J Indonesia 2001; 10:22-8)

Keywords: BPH, high PSA, PIN, proliferative activity, s-phase fraction

Benign prostatic hyperplasia (BPH) is the most common neoplastic disease in men, with the prevalence increases to nearly 100% in men aged 90 years. In Indonesia, BPH is the second leading cases found in patients visiting urology clinic.

Prostate-specific antigen (PSA) has long been known as a tumor marker of prostatic tumors. It is expressed in both BPH and adenocarcinoma involving the epithelial cells of the prostate, therefore PSA is an important tumor marker and have revolutionized the diagnosis and management of prostatic cancers today. PSA is a single-chain glycoprotein of 237 amino acids and four carbohydrate side chains. Functionally, PSA is an organ-specific, kallikrein-like, serine protease produced by prostatic epithelial cells lining the acini and ducts of the prostate gland.
Being organ specific but not disease specific; therefore it can not always differentiate BPH from adenocarcinoma of the prostate.\(^7\)

As a general consensus, clinicians have used the value of 0-4.0 ng/mL as the normal concentration of PSA. But, PSA value can increase in several circumstances, such as malignancy, inflammation, catheterization,\(^7\) and digital rectal examination,\(^5\) among which, malignancy has been the most important disease found in Western population. In the absence of prostate cancer, serum PSA is primarily derived from the transitional zone BPH and not from the peripheral or central zones.\(^8\)

During a five-year period from 1994 till 1998, in Dr. Cipto Mangunkusumo National Central General Hospital (a public hospital) and Sumber Waras Hospital (a private hospital), there were 873 patients coming to urology clinic with clinical symptoms of bladder outflow obstruction due to BPH. The mean PSA level was 12.9 ng/mL and the mean PSA-density was 0.25.\(^9\)

The high value of PSA level above the normal accepted value in our patients have risen a question whether there are other factors that might increase the PSA level or it can be suspected that there is malignancy unidentified by conventional examination. For this purpose, 3 different studies were conducted, i.e. the effect of catheterization, the presence of inflammation and pre-malignant condition such as prostatic-intraepithelial neoplasia from histopathological evaluation, and in the cellular level, the proliferative activity of BPH cells.

METHODS

Patients Inclusion

For the effect of catheterization, we retrospectively analyzed BPH patients in Dr. Cipto Mangunkusumo Hospital and Sumber Waras Hospital from 1994-1998. To evaluate the effect of co-existing abnormalities in pathological examination, we retrospectively collected data of non-catheterized patients from Dr. Cipto Mangunkusumo Hospital between 1998-1999, which were available with more details on histopathological findings. To study the proliferation activity, we obtained paraffin specimens of non-catheterized patients from the Department of Anatomical Pathology between 1994-1998, which could be found and still usable.

Physical Examination

Patients were consecutively included in our study based on the clinical examination showing prostatic enlargement. Patients' bothersomeness were scaled using Madsen-Iverson score. Diagnosis of BPH was then established after digital rectal examination (DRE), which revealed enlargement of the gland; transrectal ultrasonography (TRUS), which showed increase of prostatic volume; and uroflowmetry, which supported the evidence of urinary obstruction. The TRUS was performed using Scanner 200 (Pie Medical, The Netherlands) with 7.5 MHz. transducer; prostate size was measured using the prolate ellipse formula: volume (cm\(^3\)) = (width \times height \times length) \times 0.52. Patients showing clinically acute signs of infection were not included in the analysis. Some bladder stones might occur simultaneously with the BPH and the patients are not excluded from the analysis.

Serum PSA Examination and PSAD Calculation

Serum PSA examination was performed in two clinical laboratories in the hospitals using the same technique. Serum PSA concentration was determined using PSA Enzyme Immunoassay (EIA), IMX PSA Assay Abbott Laboratories (North Chicago, IL IRMA Count Assay, Diagnostic Product, Los Angeles, CA). Because of some conditions such as urinary retention, blood samples could be taken after a bladder catheterization and/or prostate manipulations such as DRE and TRUS. In most instances, blood samples were obtained either before DRE / TRUS or two weeks after DRE / TRUS. PSAD was defined as the quotient of serum PSA level (ng/mL) divided by prostate volume (cm\(^3\)) without unit.

Prostate Biopsy

Based on PSA examination, patients were categorized into three groups: patients with serum PSA level below 4 ng/mL, patients with serum PSA level in the intermediate range (4-10 ng/mL), and patients with serum PSA level more 10 ng/mL. Patients with PSA less than 4 ng/mL, would not undergo prostate biopsy and they were assumed to be free from cancer based on the DRE and TRUS examinations. Prostate biopsies were performed in patients with intermediate PSA level that had PSAD > 0.15 and patients with PSA level more than 10 ng/mL.
Histopathological examination

Specimens were obtained from transurethral resection of the prostate (TURP). The tissues were paraffin-fixed and stained with the routine haematoxylin-eosin (HE) staining. Evaluation of the tumor's histopathology consists of: histopathological type (nodular hyperplasia of the prostate or adenocarcinoma), the presence of prostatic intraepithelial neoplasia (PIN) or atypical adenomatous hyperplasia (AAH), the presence of inflammation and the type of inflammatory cells (acute, chronic or both).

Cell cycle analysis using flow cytometer

A section of 50 μm thickness was cut from the paraffin blocks to be analysed flow cytometrically. The paraffin sections were deparaffinized with xylene and some ethanol solution in decreasing concentration and rehydrated with distilled water according to Hedley modification. Then, the specimens were fragmented enzymatically using 0.5 ml 0.5% pepsin (Sigma, P-7012) and was filtered through 50 micron stainless steel mesh. After this, 0.5 ml of Solution B (DNA Staining Kit, Sigma) containing trypsin inhibitor and ribonuclease A for 10 minutes was added in the suspensions. Finally, they were incubated for at least 20 min. with Solution C (DNA Staining Kit, Sigma) containing propidium iodide. Cell cycle analysis were done with FAC Sort machine (Becton Dickinson, USA) using Cell Quest Page and ModFIT program under Macintosh Computer. Histogram were analysed after applying appropriate 'gate' on the dot-plot mode.

Data analysis

Description of patients, groups and subgroups are presented as tables. Descriptive statistics were developed on catheterized and non-catheterized patients, PSA groups, the presence of cancers and other histopathological findings. Analytical statistics was made between PSA subgroups and prostate volume using ANOVA test. PSA means among patients' groups, means of S-phase fraction among BPH groups and PSA subgroups was compared using the student t test. Values of p < 0.01 were considered statistically significant. The statistical analysis was performed with the statistical computer program SPSS version 10.05 for Windows.

RESULTS

Correlation between PSA values and prostate volume and catheterization procedure

From September 1994 to August 1997, there were 805 eligible cases from both Urology Clinics in Sumber Waras Hospital and Dr. Cipto Mangunkusumo General Hospital. All patients had lower urinary tract symptoms as their major complaints supported by physical examination and ultrasonography. The mean age was 66 years with the minimum age is 40 years and the oldest is 95 years old. Description of the patients was given in Table 1.

PSA values were significantly higher in patients with higher prostate volume (Table 1). Of 805 patients, majority of patients (57.3%) had urinary retention and was catheterized. In this group, the mean PSA level was significantly higher compare to the non-catheterized patients (16.3 vs. 6.8 ng/mL) (Table 2).

<table>
<thead>
<tr>
<th>PSA (ng/mL)</th>
<th>Mean Age</th>
<th>Total cases</th>
<th>Mean Prostate Volume (cc)</th>
<th>No. Biopsy</th>
<th>Prostate Cancer</th>
<th>BPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4.0</td>
<td>63 yr</td>
<td>240</td>
<td>36.9 ± 15.1*</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.1 - 10.0</td>
<td>66 yr</td>
<td>230</td>
<td>50.9 ± 22.8*</td>
<td>108</td>
<td>3</td>
<td>105</td>
</tr>
<tr>
<td>&gt; 10.0</td>
<td>68 yr</td>
<td>335</td>
<td>64.0 ± 26.3*</td>
<td>335</td>
<td>31</td>
<td>304</td>
</tr>
<tr>
<td>Total cases</td>
<td>66 yr</td>
<td>805</td>
<td>52.2 ± 25.1</td>
<td>443</td>
<td>34</td>
<td>409</td>
</tr>
</tbody>
</table>

*p = 0.000 (significant)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Prostate Volume (cm^3)</th>
<th>PSA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With retention (n = 461)</td>
<td>69.62</td>
<td>55.9 ± 1.3</td>
</tr>
<tr>
<td>Without retention (n = 344)</td>
<td>64.01</td>
<td>47.4 ± 1.3</td>
</tr>
</tbody>
</table>

*p = 0.000 (significant)
Among catheterized patients, 22 were positive of prostate cancer whereas in non-catheterized patients prostate cancer was found in 12 men (Table 3).

### Table 3. Incidence of prostate cancer within different groups of PSA level in patients with or without catheter

<table>
<thead>
<tr>
<th>PSA level (ng/ml)</th>
<th>% of cancer in patients with catheterization (n = 461)</th>
<th>% of cancer in patients without catheterization (n = 344)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 4.0</td>
<td>0/80</td>
<td>0/161</td>
</tr>
<tr>
<td>4.1-10.0</td>
<td>2/128 (1.56%)</td>
<td>1/102 (0.98%)</td>
</tr>
<tr>
<td>&gt; 10.0</td>
<td>20/253 (7.9%)</td>
<td>11/81 (13.6%)</td>
</tr>
</tbody>
</table>

### Correlation between PSA values and histopathological pattern

There were 82 non-catheterized patients with more descriptive histopathological results between 1998-1999. It was shown that there were other histopathological findings such as prostatic intraepithelial neoplasia (PIN) and histological inflammation. The overall mean PSA level of this group of patients was $5.4 \pm 0.6$ ng/ml. PSA values tend to increase above normal accepted values in pre-malignant group of patients and chronic prostatitis (Table 4).

### Correlation between PSA values and S-phase fraction

Eighty-seven thin sections of paraffin blocks from BPH patients without urinary catheterization still could be obtained from 1994-1999. Of the 87 samples, only 55 could produce satisfactory cell-cycle histograms. All samples could achieve a single $G_0/G_1$ peak with coefficient of variation below 8.0%. 36 patients had histologically confirmed BPH lesions, 19 had BPH plus PIN.

Proliferation of cells in BPH with PIN was significantly higher than those without PIN (14.1% vs. 11.2%) and among PIN positive cases there was no difference of proliferation activity between the low- and high-PSA subgroups (Table 5). In the contrary, from the 36 cases of BPH without PIN, there was a difference of proliferation activity between low-PSA group (0-4 ng/mL) and high-PSA group (> 4 ng/mL). This difference is statistically significant (Table 6).

### Table 4. Histopathological evaluation on BPH slides between 1998-1999 (n = 82)

<table>
<thead>
<tr>
<th>BPH</th>
<th>Number of cases</th>
<th>PSA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pre-malignant properties</td>
<td>59/82 (72.0%)</td>
<td>5.0*</td>
</tr>
<tr>
<td>Low-grade PIN</td>
<td>19/82 (23.2%)</td>
<td>5.2*</td>
</tr>
<tr>
<td>High-grade PIN</td>
<td>0/82</td>
<td>0</td>
</tr>
<tr>
<td>Squamous metaplasia</td>
<td>1/82 (1.2%)</td>
<td>9.3*</td>
</tr>
<tr>
<td>Atypical Adenomatous Hyperplasia (AAH)</td>
<td>3/82 (3.7%)</td>
<td>14.3*</td>
</tr>
</tbody>
</table>

*p (significance) = 0.031

### Table 5. Percentage of S-phase fraction in low and high PSA group of BPH patients with or without PIN

<table>
<thead>
<tr>
<th>PIN (+)</th>
<th>PIN (-)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA ≤ 4 ng/ml</td>
<td>13.4 ± 2.8 % (n = 6)</td>
<td>8.9 ± 4.2 % (n = 16)</td>
</tr>
<tr>
<td>PSA &gt; 4 ng/ml</td>
<td>14.3 ± 4.7 % (n = 13)</td>
<td>13.1 ± 4.4 % (n = 20)</td>
</tr>
<tr>
<td>Total</td>
<td>14.1 ± 4.1 %* (n = 19)</td>
<td>11.2 ± 4.7 %* (n = 36)</td>
</tr>
</tbody>
</table>

*not significant (p = 0.566), *significant (p = 0.033)
Table 6. Percentage of S-phase fraction in low and high PSA group of BPH patients without PIN

<table>
<thead>
<tr>
<th></th>
<th>BPH without PIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA ≤ 4 ng/ml (n = 16)</td>
<td>8.9 ± 4.2 % * (range: 2.7 - 16.7 %)</td>
</tr>
<tr>
<td>PSA &gt; 4 ng/ml (n = 20)</td>
<td>13.1 ± 4.4 % * (range: 10.0 - 22.7)</td>
</tr>
</tbody>
</table>

*significant (p = 0.008)

DISCUSSION

In Indonesia, until recently prostate cancer had not been much of a problem, though in the last 10 years there has been an increase in cancer incidence. This has led to increasing interest in use of PSA testing, though its sensitivity and specificity are still debatable.

Nevertheless, until today serum PSA level is still an important tool for diagnostic screening of prostate cancer. An increase of more than 4.0 ng/ml is indicative to perform additional diagnostic procedure to find prostate cancer. But, there is still a problem in selecting patients to be the candidates of prostate needle biopsy, i.e. if they clinically show signs and symptoms of BPH and normal consistency of DRE. The fact that all screening modalities including serum PSA level are not specific will cause too many unnecessary biopsies. This condition will increase not only the logistic burdens, but also morbidity and psychological aspects of the patient. Beside the low-specificity, serum PSA level could also increase in some circumstances which are not related to malignancy, such as DRE-procedure, TRUS, prostate massage, prostatitis and some instrumentation procedures like cystoscopy, indwelling catheterization and TURP.

Carcinoma of the prostate is a very important malignant disease in the United States. In 1996, prostate cancer was 40% of all new malignant diseases in men and gave 14% mortality due to malignant disease in men. In Indonesia on the other hand, based on histopathological data between 1988-1990, prostate cancer was included in the ten highest malignancy in men, i.e. the seventh in 1988, the ninth in 1989 and back to seventh again in 1990. Although in Jakarta prostate cancer was ranked as the second highest cancer of genitourinary system, the number of cases is relatively very low compared to the incidence in the United States and European countries.

In our country, there was a tendency that most BPH patients came in already severe conditions; they had to be catheterized in the emergency room prior to further examinations. This condition is unavoidable since most patients came from low-economic and low-education group of people, so they had never been aware of the initial symptoms of BPH. Catheterization in BPH patients has been known to raise the PSA concentration and might denote different strategy of interpreting PSA. Our data showed that catheterization increased the PSA level 2.4 times above the normal value (Table 2). It was also obvious that increase PSA level could be due to high prostate volume; this is a constant finding of our reports.

Prostatitis is the commonest known cause of false positive PSA in the West. According to some reports, clinically detectable prostate cancer accounts for only 34% of serum PSA elevations, while nearly all of the men with high PSA concentrations and the majority with normal levels had at least one biopsy specimen coded positive for chronic inflammation.

Inflammation was defined as inflammatory cells in the prostatic stroma, and could be acute and/or chronic inflammation. Inflammation of the prostate is also a common histological finding in prostate biopsies. This subclinical inflammation can cause PSA elevation. Our study has shown that all patients without urinary retention had histological inflammation, mostly chronic infections. Although there was no control, it could be seen that the PSA tend to be higher than normal accepted values.

Inflammation most probably increases serum PSA concentrations by causing leakage of PSA from the acini and ductal lamina, since prostate duct integrity is disturbed. Itani et al found that acute inflammation increased serum PSA more than subclinical inflammation. They concluded that unless associated with glandular epithelial disruption, density of prostatic interstitial inflammatory cell infiltrate is not significantly correlated with serum PSA concentration. All histological section comprised a mononuclear cell infiltrate.

In this current report, more descriptive histopathological report was not available before 1998. Evaluation on recent data has shown that PIN and other pre-malignant findings could increase the PSA level. The low significance of our data was due to
The attempt to correlate proliferative activity and PSA level is relatively new in BPH study. Increase of prostate volume could be due to either an increase in cell proliferation with unchanged cell death or unchanged cell proliferation with lower cell death. Some studies have shown that induction of BPH from normal prostate is obviously associated with a distinct increased proliferation of epithelium and stroma, and further increase in BPH volume, however, is not correlated with a further increase in proliferation. It's should be noted that PSA is produced by normal prostatic cells as well as hyperplastic and malignant cells.

Our study showed that tumors with elevated serum PSA level have higher proliferative activity as indicated by the percentage of S-phase cells. There is no universal acceptance for S-phase cutoff point, but in most cycling human cell population, the nuclei in the S- and G2/M phases may reach 15%. As comparison, proliferation or S-phase fraction rates of breast cancer has been characterized as: <8% = low proliferation, 8-12% = intermediate proliferation, and > 12% = high proliferation. But this is also not a uniform agreement. Using this classification as an example, our patients with low PSA level could be categorized as intermediate proliferation and the patients with high PSA level as high proliferation. High proliferation was found in patients with high PSA as well as BPH with PIN (Table 5). High-grade PIN was frequently associated with high cancer risk, therefore it is not surprising if this cases had high proliferation index. However, the association of high S-phase fraction and high PSA level in the absence of PIN suggests that there was an increased mitogenic process in these patients. It is not known what mechanism that could increase the mitogenic activity.

CONCLUSION

As conclusion, our study showed that the high serum PSA level of our patients was primarily caused by urethral catheterization and increased prostate volume. Although the sample size was small and lack of high-grade PIN, subclinical inflammation and PIN tend to raise PSA level above normal accepted value. Cases with high PSA also associated with high proliferative activity, which is suggestive to mitogenic process indicating early neoplastic process or inflammation. Further study is needed to elaborate more details at the cellular and molecular level.

REFERENCES