TAJ June 2009; Volume 22 Number 1

ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

Free PSA is A Better Tumor Marker Than Serum Total PSA in Diagnosis of Prostatic Carcinoma

S M Asafudullah[1], M A Salam[2], S M Badruddoza[3], M Khalilur Rahman[4]

Abstract

Estimation of serum prostate specific antigen (PSA) has been considered as valuable non-invasive biochemical diagnostic tool for early detection of prostatic carcinoma. This cross sectional purposive study was carried out to compare the performance of serum total PSA and free PSA in terms of their sensitivity, specificity and overall diagnostic accuracy for prostatic carcinoma among clinically suspected cases. This study included a total of fifty (50) DRE (digital rectal examination) positive patients admitted in Rajshahi Medical College Hospital (RMCH) and Private hospitals in Rajshahi city during the period of January, 2006 to January, 2008. Estimation of serum total PSA and free PSA were done by ELISA (Enzyme linked immunosorbent assay) method using commercially available kits and histopathological examination of the surgically resected prostatic tissue was done for laboratory confirmation of the diagnosis of prostatic carcinoma for all patients. Diagnostic sensitivities, specificities and overall accuracy of serum total PSA and free PSA were calculated using standard formulae against histopathological diagnosis. Prostatic carcinoma was detected by histopathological examination in 41 cases out of 50 patients with the mean age of 71.2 ± 10.1 years. The sensitivity, specificity and overall diagnostic accuracy of serum total PSA (at cut off value of ≥10 ng/ml) were found to be 80.48%, 88.90% and 82.00% respectively while they were 92.68%, 77.80% and 90.00% respectively for serum percent free PSA (at cut off value of ≤25%). It is inferred that percent free PSA is a better tumor marker than serum total PSA in the diagnosis of prostate carcinoma.

TAJ 2009; 22(1): 30-35

Introduction

Enlargement of prostate gland due to tumor and other causes are old age ailments causing significant morbidity and mortality. Nodular hyperplasia of prostate (NHP) and prostatic carcinoma are the two major entities affecting the human prostate. Prostate cancer is among the most frequently encountered male cancers in any population. It accounts for 33% of all malignant tumors in men and responsible for 9% of all deaths due to cancer[1]. Carcinoma of the prostate is the most common form of cancer in men in the United States and is responsible for 10% of cancer death in this population[2]. In Bangladesh, the annual report of National Institute of Cancer Research and Hospital, 2005 has documented the incidence of prostate carcinoma as 1.2% among all cancers[3].

[1] Assistant Professor, Department of Pathology, Rajshahi Medical College, Rajshahi.
[2] Associate Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.
[3] Professor, Department of Pathology, Rajshahi Medical College, Rajshahi.
[4] Assistant Professor, Department of Medicine, Rajshahi Medical College, Rajshahi

For more than a half century, a serum acid phosphatase has been considered as the gold standard for the diagnosis of prostate carcinoma[4]. However, in 1979, Wang and his colleagues at Rosevell Park, seeking a better marker for prostate cancer, isolated prostate-specific antigen (PSA) from prostate tissue. Prostate-specific antigen is a chymotrypsin like serine protease produced and secreted at high concentration into seminal fluid where its role is to dissolve the gel that forms after ejaculation. Its use as a marker for prostate cancer is based on the fact that only small amount of PSA leaks from the normal prostate into the circulation, thus the serum concentration are normally very low than those in seminal fluid. But disruption of basal cell layer and basement membrane covering the prostate gland by any condition allows PSA in the circulation with consequent elevated level. In general, PSA level below 4 ng/ml have a low probability of having clinically detectable prostatic cancer. PSA level above 4 ng/ml is considered abnormal. Level of 4-10 ng/ml is a diagnostic gray zone and level higher than 10 ng/ml strongly suggests prostatic cancer. Since then estimation of serum total prostate–specific antigen (PSA) has been considered as very important tumour marker for prostatic carcinoma. But as a diagnostic dilemma, PSA level has also been found elevated in benign prostatic conditions especially with nodular hyperplasia, which is a common occurrence in men aged over 50. Therefore, attempts have been made to improve its diagnostic specificity. Estimation of percent free prostate specific antigen (free PSA/total PSA X 100) is an improvement of original PSA level and considered as better non-invasive diagnostic tools for prostatic cancers[5, 6]. Percent free PSA is lower in prostatic carcinoma than other benign prostatic conditions. Further, determination of percent free PSA level has been found to be most valuable in cases of diagnostic gray zone of PSA level. Percent free PSA of >25% indicate lower risk of cancer while the level of ≤25% is suggestive of prostatic cancers[7].

Histopathological changes detected on biopsies or resected prostatic tissue is the hallmark for the diagnosis of prostatic carcinoma. However, the facility for histopathological examination is not widely available and it is also time consuming. Considering the importance of the biochemical parameters for the early diagnosis as well as for adopting better treatment strategy for prostatic carcinoma patients, the present study was undertaken to compare the diagnostic sensitivities, specificities and accuracy of serum total PSA and percent free PSA among suspected prostatic carcinoma cases.

Material and Methods

Patients

Fifty (50) clinically suspected and DRE-positive patients of prostatic carcinoma of age ≥ 50 years those who were admitted at Rajshahi Medical College Hospital (RMCH) and different private hospitals of Rajshahi city of Bangladesh during January, 2006 to January, 2008 were included.

Selection criteria of patients

Symptoms of prostatism such as frequency, urgency, hesitancy, decrease flow, incontinence and nocturia, abnormal nodule and /or fixed prostate on DRE and any sign of metastasis detected by clinical, radiological or biochemical examinations were considered for inclusion criteria. While, patients with lower urinary tract symptoms of long duration, palpable prostate with smooth mucosal border on DRE, patients having prostatic calculi or abscess detected in ultrasonography. and symptomatic prostatism improved by conservative treatment was excluded from the study.

Sample collection

After informed written consent, a single sample of 5.0 ml venous blood was collected before DRE, using disposable syringe and needle from each patient after disinfecting the selected venipuncture site with 70% alcohol. The collected blood was immediately transferred into a sterile test tube without any anticoagulant for separation of serum. About 30 minutes after blood collection, the test tube was centrifuged at 4000 rpm for 20 minutes and using tip of micropipette, 1.0 ml of serum was collected from the top of the centrifuged deposit. The serum was preserved into a 1.5 ml microcentrifuge (eppendrof) tube for ELISA.

Laboratory procedures

Enzyme linked immunosorbent assay (ELISA) for the quantitative determination of serum total PSA and free PSA

ELISA was performed by Ex 808 Multiskan ELISA reader for all 50 cases following standard procedures for quantitative estimation of PSA.

Principle and Procedure of the total PSA test: The ELISA for estimation of PSA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit anti-PSA antibody directed against intact PSA for solid phase immobilization (on the microtiter wells). A monoclonal anti-PSA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample was allowed to react first with the immobilized rabbit antibody at room temperature for 60 minutes. The wells were washed to remove any unbound antigen. The monoclonal anti-PSA-HRP conjugate was then reacted with the immobilized antigen for 60 minutes at room temperature resulting in the PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells were washed with water to remove unbound-labeled antibodies. A solution of TMB reagent was added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition of stop solution changing the color to yellow. The concentration of PSA is directly proportional to the color intensity of the test sample. Absorbance was measured spectrophotometrically at 450 nm.

Principle and Procedure of free PSA (f-PSA) test: The f-PSA ELISA test is a solid phase two-site immunoassay. An anti-f-PSA monoclonal antibody is coated on the surface of the microtiter wells and a rabbit anti-PSA antibody labeled with horseradish peroxidase is used as the tracer. The f-PSA molecules present in the standard solution or sera are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen complex, the unbound antibody-enzyme tracers were removed by washing. The horseradish peroxidase activity bound in the wells was then assayed by a colorimetric reaction. The intensity of the color formed was proportional to the concentration of f-PSA present in the sample.

Histopathological examination

After surgical operation, resected prostatic tissue from all cases was preserved in 10% buffered formalin. Then tissue was transported to the Department of Pathology, Rajshahi Medical College for histopathological examination. After tissue processing and paraffin embedding, 4-5 µm thickness serial section was made; one slide was made from each block and stained with Heamatoxylin and Eosin (H&E). The slide was examined under light microscope and the findings were noted.

Statistical analysis

The sensitivity and specificity were calculated by using the following formulae: *Sensitivity* = [number of samples with true-positive results / (number of samples with true-positive results + number of samples with false-negative results)] x 100; *Specificity* = [number of samples with true-negative results / (number of samples with true-negative results + number of samples with false-positive results)] x 100. The positive and negative predictive values were calculated as the number of samples with true-positive results/ (number of samples with true-positive results + number of samples with false-positive results) and as the number of samples with true-negative results/ (number of samples with true-negative results + number of samples with false-negative results), respectively. *Diagnostic accuracy* = [number of samples with true-positive results + number of samples with true-negative results / samples with true-positive results + number of samples with false-positive results + number of samples with false-negative results + number of samples with true-negative results x 100.

Results

Table-I shows the distribution of patients into different age groups and the frequency of prostate cancers among different groups. It is evident that

42% of the patients were between 71 – 80 years old with 100% cancer, followed by 30% between 61 – 70 years with 46.66% cancer, 22.00% above 80 years with 90.90% cancers and 6.00% up to 60 years of age with 100% prostatic cancers. The mean age of the patients was 71.2 ± 10.1 years, with age ranging of 50-95 years.

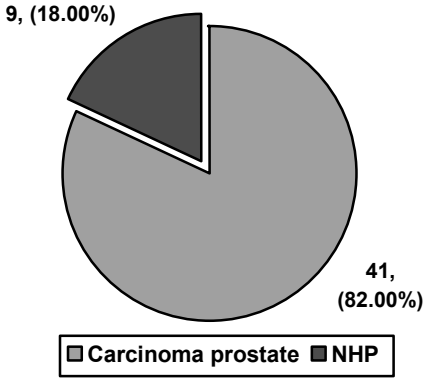
Distribution of prostatic carcinoma cases as evidenced by histopathological examination of resected prostatic tissue among clinically suspected patients is shown in Figure-1.

Table-I: Distribution of the patients by age and frequency of prostate cancers (n = 50)

Age (years)	Number of patients	Frequency of prostate cancers
Up to 60	03 (06.00)	03 (100)
61 – 70	15 (30.00)	07 (46.66)
71 – 80	21 (42.00)	21 (100)
Above 80	11 (22.00)	10 (90.90)
Total	50 (100)	41 (82.00)

Figures in the parenthesis indicate percentage

Figure-1: Histopathological findings of resected prostatic tissue



NHP: Nodular hyperplasia of prostate

Correlation of serum total PSA level with prostatic carcinoma is shown in Table-II. Overwhelming majority (97.00%) of the prostatic carcinoma patients had serum total PSA level of >10 ng/ml, which has been taken as the cut-off value.

Table-III shows the levels of percent free PSA and its correlation with prostatic carcinoma. 95% prostatic carcinoma patients had percent free PSA of ≤25 against only 30% with percent free PSA of >25.

Table-II: Serum total PSA level and frequency of prostatic carcinoma (n = 50)

Total serum PSA (ng/ml)	Number	Frequency of Cancer
< 10	16 (32.00)	08 (50.00)
≥ 10	34 (68.00)	33 (97.00)
Total	50 (100)	41 (82.00)

(Figures in the parenthesis indicate percentage)

Table-III: Percent free PSA and its correlation with prostatic carcinoma (n = 50)

Level of % free PSA	Number of cases	Number of cancer
≤25	40 (80.00)	38 (95.00)
>25	10 (20.00)	03 (30.00)
Total	50 (100)	41 (82.00)

(Figures in the parenthesis indicate percentage)

The sensitivity, specificity and over all diagnostic accuracy of serum total PSA and free PSA are summarized in Table-IV. It is evident from the table that, the sensitivity (92.68% vs. 80.48%) and the over all diagnostic accuracy (90% vs. 82%) of free PSA were better than total PSA.

Table-IV: Sensitivity, specificity and diagnostic accuracy of serum total PSA and free PSA for prostatic carcinoma

Tests	Sensitivity	Specificity	Diagnostic Accuracy
Serum total PSA (Cut-off value >10 ng/ml)	80.48%	88.90%	82.00%
Percent free PSA (Cut-off value ≤25%)	92.68%	77.80%	90.00%

Legend and caption of Tables and Figure

Table-I: Distribution of the patients by age and frequency of prostate cancers (n = 50)

Figure-1: Histopathological findings of resected prostatic tissue

Table-II: Serum total PSA level and frequency of prostatic carcinoma (n = 50)

Table-III: Percent free PSA and its correlation with prostatic carcinoma (n = 50)

Table-IV: Sensitivity, specificity and diagnostic accuracy of serum total PSA and free PSA for prostatic carcinoma

Discussion

Although potentially curable, prostate cancer produces no symptoms for long time and majority of the patients seek medical attention only after the onset of symptoms related to advanced or metastatic disease. This accounts for high rate of mortality from prostate cancers. After 50, age-specific incidence rate of prostate cancer increases three or four folds for every 10 year increase in age[8] (Table-I) . Recently, the avenue for the diagnosis of carcinoma prostate has been enhanced by discovery of PSA as a screening tumor marker. PSA is the first organ-specific serum marker in all of cancer biopsy and clearly is the most important tumor marker for carcinoma of the prostate.

Determination of serum free PSA for calculation of percent free PSA is more acceptable biochemical diagnostic parameter for carcinoma prostate with increased sensitivity and specificity than total PSA. In the present study, both serum total PSA and free PSA have been determined for all patients in order to compare their performance in the diagnosis of prostatic carcinoma. Both the biochemical parameters showed very good range of sensitivity, specificity and overall diagnostic accuracy when compared with histopathological findings, which is the gold standard of diagnosis. But free PSA was found to be a better tumor marker in terms of its sensitivity and diagnostic accuracy than total PSA. It was noted that 95.00% patients having prostate carcinoma had percent free PSA of ≤ 25% patients, while only 30.00% patients had prostate carcinoma with percent free PSA at the cut-off value of >25% (Table-III). The frequency of prostatic carcinoma with ≤ 25% cut-off value of percent free PSA was found as 95% in different studies[9,10,11] and our findings are well consistent with them. Different cut-off values for percent free PSA ranging from <10 to 25% for the diagnosis of prostate carcinoma have been used by different authors with varying but acceptable sensitivity and specificity[9,12]. Estimation of percent free PSA has been claimed to be superior over total PSA by many authors stating that it can minimize the problem of diagnostic gray zone i.e. PSA level between 4 to 10 ng/ml in case of total PSA . Although serum total PSA level >10 ng/ml usually has good correlation with prostatic carcinoma which has been noted in the present study (97%) as well but its diagnostic significance is much variable in cases of diagnostic gray zone[13]. The present study has revealed that 50% of prostatic carcinoma patients had their serum total PSA level < 10 ng/ml. In patients with marginally elevated PSA, the percent free PSA can decrease the biopsies by 81-85% (Catalon et. al., 1995). Further, using Chi-square test, it was found that, the percent free PSA can differentiate nodular hyperplasia of prostate (NHP), the most common benign condition with elevated PSA level from carcinoma of prostate significantly ($p<0.001$) [14] .

The sensitivity, specificity and diagnostic accuracy noted for biochemical parameters in the present study (Table-IV) are in accordance with findings of others[15,16]. The diagnostic accuracy found for each of these tests is quite encouraging for its use in the diagnosis of prostate carcinoma. But in comparison, the diagnostic accuracy of percent free PSA has superseded the accuracy of total PSA and that is why this test is claimed to be superior over total PSA in the recent studies.

In conclusion, it can be said that periodic check-up by DRE and estimation of serum PSA in particular percent free PSA after 50 are good practices adopted in many developed countries as a routine for evaluation of prostatic cancer with the view to diagnose it much earlier to have better prognosis for therapeutic management.

Reference

1. Fletcher CDM. 'Tumors of the male genital tract'. Diagnostic Histopathology of tumors. 3rd edition, Philadelphia, Elsevier, 2007: 755.
2. Lew EA, Garfinkel L. Mortality at ages 75 and older in the cancer prevention study (CPSI). Cancer. 1990; 40: 210.
3. Talukder H, Jabeen S, Shaheen S, et al. Hospital Cancer Registry, National Institute of Cancer Research and Hospital, Annual Report 2005.
4. Ishibe T, Usui T, Nihira H. Prognostic usefulness of serum acid phosphatase levels in carcinoma of the prostate. J Urol. 1994; 112: 237-240.
5. Wolff JM, Borchers H, Efferts PJ, Habid FK, Jakse G. Free to total prostate-specific antigen serum concentrations in patients with prostate cancer and benign prostatic hyperplasia. Br J Urol. 1995; 78: 409-413.
6. Brassell SA, Kao T, Sun L, Moul JW. PSA versus PSAD as predictor of tumor volume, margin status, pathologic stage and biochemical recurrence of prostate cancer. Urology. 2005; 66: 1229-1233.
7. Stephen J. Freedland, Partin AW. Prostate-specific antigen: Update 2006. Urology. 2006; 67**:** 458-460.
8. Song JM, Kim C, Chung HC et al. 'Prostate specific antigen, digital rectal examination and transrectal ultrasonography : a meta-analysis for this diagnostic triad of prostate cancer in symptomatic Korean men'. Yonesi Medical Journal, 2005; 46: 414-424.
9. Chen Z, Chen H, Stamey TA, Stanford CA. Prostate specific antigen (PSA) in benign prostatic hyperplasia (BPH) tissue is different from PSA in prostate cancer tissue. J Urol. 1996; 155: 696.
10. Ozen H, Sozen S. PSA Isoforms in Prostate Cancer Detection. *European* Urology Journal. 2006; 5: 495-499.
11. Catalona WJ, Smith DS, Wolfert RL, Wang TJ, Rittenhouse HG, Ratliff TL. Increased specificity of PSA screening through measurement of percent free PSA. J Urol. 1995; 153 (Suppl 1): 312.
12. Luderer AA, Chen YT, Soriano TF, et al. Measurement of the proportion of free-to-total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. Urology. 1995; 46: 187-194.
13. Christensoon A, Bjork T, Nilsson O, Duhlen U, Matikainen MT, Cockett ATK et al. Serum prostate specific antigen complexed to x-antichymoerypsin as an indicator of prostate cancer. J Urol. 1993; 150: 100-150.
14. Prestigiacomo AF, Stamey TA. Can free and total prostate specific antigen and prostate volume distinguish between men with negative and positive systematic ultrasound guided prostatic biopsies. J Urol. 1997; 157: 189-194.
15. Akdas A, Tarcan T, Turkeri L, Cevik I, Biren T, Gurmen N. The diagnostic accuracy of digital rectal examination, transrectal ultrasonography, prostate specific antigen, and PSA density in prostate carcinoma. Br J Urol..1995; 76**:** 54-56.
16. Gillatt D, Reynard JM. What is the "normal range" for prostate-specific antigen? Use of a receiver operating characteristic curve to evaluate a serum marker. Br J Urol. 1995; 75: 341-346.

All correspondence to:
S M Asafudullah
Lecturer
Department of Pathology
Rajshahi Medical College, Rajshahi.