

The Comparison of Percent Free PSA with total PSA in the diagnosis of Prostate Cancer

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Abstract

Objective: To determine the ratio of free to total PSA in patients' of carcinoma prostate and nodular hyperplasia, and to compare this ratio with total PSA for diagnosis of prostate cancer.

Settings: The study was conducted in Armed Forces Institute of Pathology (AFIP) Rawalpindi in collaboration with Armed Forces Institute of Urology (AFIU) Rawalpindi, over a period of 7 months (February 2000 to August 2000).

Materials and Method: One hundred and seventy nine blood samples were collected from patients admitted for prostatectomy. After surgery, diagnosis of prostate carcinoma or nodular hyperplasia was confirmed histologically. Serum samples were analyzed for total and free PSA.

Results: The $\pm$ 2SD values of total PSA for nodular hyperplasia were 4.07-13.93 ng/ml (mean = 9.00 ng/ml), and for carcinoma prostate were 9.34-23.74 ng/ml (mean = 16.54 ng/ml). These values showed a considerable overlap. While 95 % confidence interval values of percent free PSA for nodular hyperplasia were 14.00 - 28.08% (average = 21.04%), and for carcinoma prostate were 2.69-16.05% (average = 9.37%) and these values showed a little overlap. The sensitivity of percent free PSA in differentiating between prostate carcinoma and nodular hyperplasia was 96% with a cut off limit of 15% of % free PSA as compared to 74 % with total PSA alone.

Conclusion: Percent free PSA is a better marker for differentiating patients of carcinoma prostate from nodular hyperplasia as compared to total PSA (JPMA 53:233;2003).

Introduction

Carcinoma prostate is the most common malignancy in males older than 50 years of age, and second most common cause of cancer deaths in United States.¹ To decrease the mortality rate of prostate cancer, it is mandatory to diagnose the disease at an early stage, when it is organ confined, so that the disease can be cured by radical surgery.² Different modalities are used in clinical practice for the diagnosis of prostate cancer. They include digital rectal examination, transrectal ultrasonography, serum prostate specific antigen and prostate biopsy.³

Prostate specific antigen (PSA) is a glycoprotein with a molecular weight of 33-34 kD. It is a serine protease, produced predominantly by the epithelial cells of ducts and acini of the prostate gland.⁴ In the seminal fluid, PSA cleaves seminal vesicle specific proteins into several very low molecular weight proteins, as a part of the process of liquefaction of the seminal coagulum. In the seminal fluid PSA can be fractionated into five isoforms.⁵ The isoforms A and B are enzymatically active, whereas isoforms C, D and E are inactive. Low levels of PSA in serum of healthy individuals are because of minimal leakage of this protein into the blood. Elevated levels are seen in patients with nodular hyperplasia of prostate, adenocarcinoma of prostate and prostatitis as well as after manipulating prostate gland by digital rectal examination, transrectal ultrasonography, catheterization, prostate biopsy and after radical

prostatectomy.⁶ After leakage into the blood, the enzymatically active isoforms of PSA, combine with serum antiproteases like α -1 antichymotrypsin (α -1 ACT) and α -2 macroglobulins (α -2 MG)⁷, where as the enzymatically inactive forms remain free in the blood which can be detected by available free PSA immunoassays.⁸⁻¹⁰

Prostate specific antigen is currently the most promising tumor marker for prostate cancer. But, PSA testing alone is not competent enough in screening and detecting early prostate cancer.¹⁰ Nodular hyperplasia of prostate is a common occurrence in men with same age group, which does cause elevation in serum PSA level. Unfortunately the overlap of PSA values in nodular hyperplasia and early carcinoma of prostate is so extensive, that selecting an optimum cutoff value of total PSA is almost impossible.¹¹ In order to optimize the use of PSA for detection of prostate cancer various concepts have evolved. These include PSA density, PSA velocity, age specific reference ranges and percentage of free PSA.¹²

Among the various modalities, the percentage of free PSA has shown potentially better results. It is proposed that the proportion of serum free PSA is significantly higher in patients with nodular hyperplasia than in patients of prostate cancer. The cancer cells, due to structural changes in PSA molecule, may produce lower proportion of free

PSA in patients of carcinoma prostate.¹³ A study was planned to evaluate the diagnostic sensitivity and specificity of percent free PSA ratio to total PSA in differentiating between nodular hyperplasia and adenocarcinoma of prostate.

Materials and Methods

This was an observational study carried out in Department of Chemical Pathology and Endocrinology in collaboration with Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi.

The patients admitted in Armed Forces Institute of Urology (AFIU), Rawalpindi, between February 2000 and August 2000 for prostatectomy were selected for the study. Patients who were catheterized, those who had digital rectal examination performed in last 2-3 days before taking blood sample, and patients suffering from urinary tract infection were excluded from the study. Venous blood sample (5 ml) was collected in a plain Venuject tube (without anticoagulant), avoiding hemolysis from each patient for total and free PSA estimation. Serum was separated from the clotted blood and stored frozen at -20⁰ C till analyzed. The detailed clinical history was recorded, physical examination and ultrasound findings and results of other investigations were noted. The time interval between sampling and performing the test ranged between 1 and 3 months. After surgery, diagnosis of prostate cancer or nodular hyperplasia was histologically confirmed.

Serum free PSA and total PSA estimations were done by a solid phase two-site chemiluminescent enzyme immunometric assay on Immulite Automated Analyzer (DPC-USA), using Immulite free PSA (Cat. Number-LKPF 1) and total PSA (Cat. Number-LKPS 1) reagent kits, duly approved by FDA of USA.

Subjects were grouped into those having nodular hyperplasia and the ones with carcinoma of prostate.

Results

A total of 179 patients were studied. Out of which 129 had nodular hyperplasia and 50 were suffering from carcinoma prostate. The average age of patients with nodular hyperplasia was 71.5 years (53-90 year), where as the average age of patients having carcinoma of prostate was 67.2 years (51-80 years).

The results of total PSA for all these patients showed that the mean value in both groups to be well apart. Calculation of 95% confidence interval (± 2 SD = 4.93) reflected significant overlap between these two groups. The histogram of total PSA (Figure 1) showed that this overlap is considerable, particularly in the zone ranging from 5.0-20 ng/ml, consisting of majority of patients.

The results of percent free PSA showed that the mean values are more spaced out between these two subgroups and the 95% confidence interval (± 2 SD = 10.12)

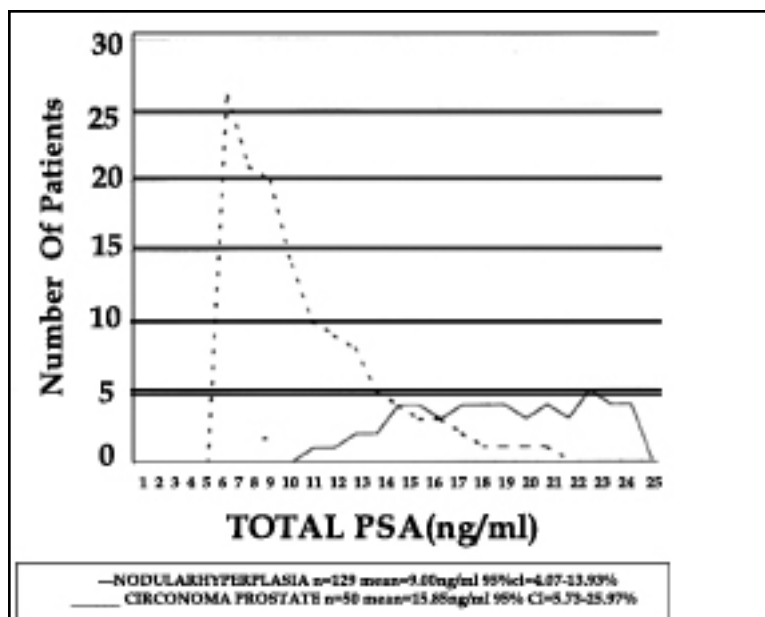


Figure 1. Frequency distribution of total PS (n=179).

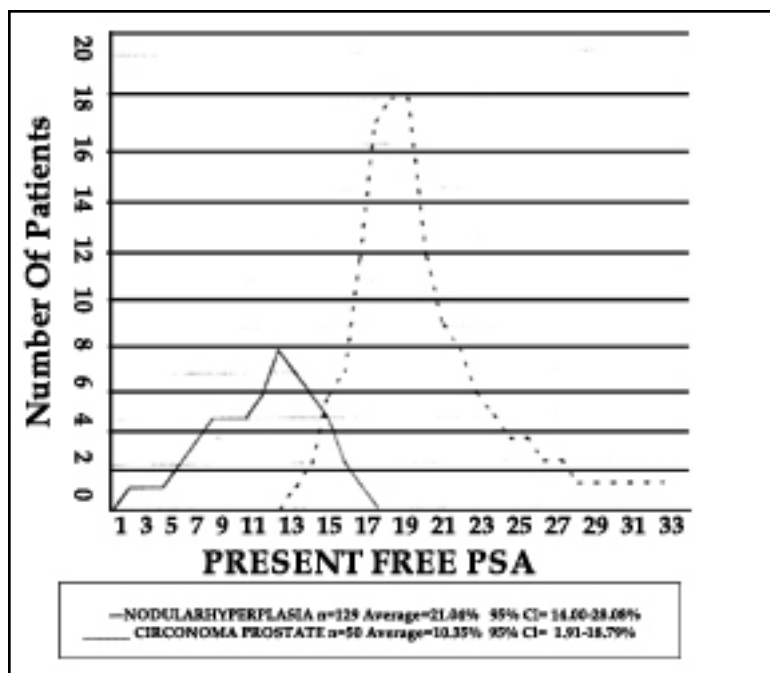
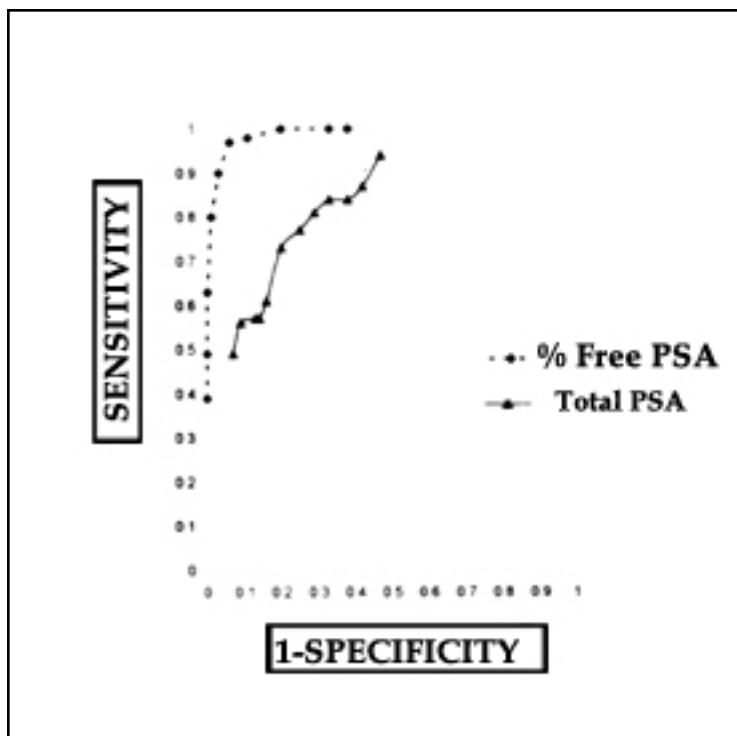


Figure 2. Frequency distribution of percent free PSA (n=179).

showed little overlap. The histogram of percent free PSA showed that the overlap of patients of nodular hyperplasia and carcinoma prostate is very little.

To determine the diagnostic accuracy of the total and free PSA in subjects with carcinoma prostate, Receiver Operating Characteristic (ROC) curves were plotted (Figure 3) showed that by additional testing with free PSA, the sensitivity rate of detecting carcinoma prostate increased from 74% to 96%, without any decrease in specificity, at a cutoff limit of 15% of percent free PSA.



Discussion

The most important advancement in enhancing the performance of prostate specific antigen, as a tumor marker is the discovery of various molecular forms of prostate specific antigen.¹⁴

In our study the comparison of mean of total PSA for 179 patients of nodular hyperplasia and carcinoma prostate shows quite a significant difference between these two groups. But the overlap of total PSA values encompassing 95% confidence intervals of these two groups is quite extensive. Majority of our patients fall in the zone ranging from 9-19 ng/ml, and in this zone selecting a cutoff value of total PSA, to differentiate between these groups seems more or less impossible. However if a cutoff level at 12 ng/ml is established, the sensitivity of total PSA to detect carcinoma prostate becomes 74%, which is not an appreciable sensitivity to detect carcinoma prostate. Christensson et al.¹⁵ in a study calculated the sensitivity of total PSA to detect prostate cancer as 66%. Again, it is a low sensitivity rate to detect prostate cancer.

Percent free PSA (proportion of free PSA to total PSA), has been shown in various studies to be more useful than total PSA in distinguishing prostate cancer from nodular hyperplasia, in patients where the total PSA levels are inconclusive.¹⁶⁻¹⁸ The main aim of almost all of them

was, to reduce the number of unnecessary prostate biopsies in individuals with slightly elevated total PSA values, and at the same time improving the sensitivity of this tumor marker for early detection of prostate cancer. Our study also shows that the percentage of free PSA discriminates better between carcinoma prostate and nodular hyperplasia than total PSA.

Similar results are found in various other studies. Christensson et al.¹⁵ evaluated free PSA and total PSA in 121 patients of prostate cancer and 144 patients of nodular hyperplasia. The percentage of free PSA was significantly lower in patients with prostate cancer relative to those with nodular hyperplasia. They established a cutoff limit of 18% for percent free PSA, which showed a sensitivity of 90% to detect early carcinoma prostate. These results are in agreement with our study that reflected a sensitivity of 96%, despite the fact that we have lowered the cutoff limit to 15%.

In another study Lilja et al.⁷ showed, that percentage of free PSA is considerably low in patients with prostate cancer than in nodular hyperplasia. His cancer patients had 18% average free PSA as compared to 28% average free PSA in patients with nodular hyperplasia. Catalona et al.¹⁹ performed a multi-center trial in which he evaluated men who had total PSA ranging from 4.0 - 10.0 ng/ml and a negative digital rectal examination, who underwent ultrasound guided needle biopsy of prostate gland. A total of 773 individuals were studied, out of which 379 (19%) were ultimately found to have cancer. He showed that total PSA was higher in patients of cancer, whereas the free to total PSA ratio was significantly higher in men with negative biopsies.

In our study Receiver Operating Characteristics curves (ROC curve) demonstrated that the sensitivity of percent free PSA in differentiating between prostate cancer and nodular hyperplasia improved to 96%, when a cutoff limit was made at 15% of percent free PSA. This free to total PSA ratio demonstrated a highly significant improvement over the total PSA concentration alone in discriminating between patients with nodular hyperplasia and prostate cancer. Similar results were found in a study by Lauderer et al.¹⁸, who showed that with a cutoff limit of percent free PSA at 20%, the sensitivity to detect carcinoma prostate increased to 88%. These observations support the concept that there is lower free to total PSA ratio in the serum of prostate cancer patients, when compared with patients of nodular hyperplasia. However the cutoff value of free PSA, that yields maximum sensitivity, varies in different studies. This difference in percentage of free PSA has been observed because of variations in study design, data analysis and various factors known to directly influence serum PSA levels such as digital rectal examination, ejaculation, exercise, catheterization and different drugs.²⁰ A number of analytical problems are also of concern, such as the fact that PSA assays developed by various manufacturers may differ in serum PSA level determination.²¹ Differences between manufacturers would be multiplied when these two analytes are measured to obtain the ratio of the free to total PSA. In our study we used both free PSA and total PSA kits that were approved by FDA of USA.

These variations in assays clearly pose problems in determining the free to total PSA ratio. Laboratories must provide information with respect to which free and total PSA assay they utilize, as well as to provide meaningful risk assessment for carcinoma prostate at a given ratio. PSA has truly revolutionized the management of men with prostate cancer. Its role in screening and early detection is both rational and unprecedented. Undoubtedly, new approaches for interpreting PSA levels in the individual patient will be discovered and new markers will be identified to aid clinicians in the diagnosis of prostate cancer.

Conclusions

Total serum PSA level cannot significantly distinguish between nodular hyperplasia and carcinoma prostate in patients having total PSA ranging from 5-20 ng/ml. By doing additional testing with free PSA and calculating percentage of free PSA, the sensitivity of prostate specific antigen as a tumor marker is enhanced to 96% for detection of prostate cancer when cutoff limit of 15% is selected.

References

1. Brawer MK. Prostate specific antigen: Current status. *CA Cancer J Clin* 1999; 49: 264-81.
2. Henricks WH, England BG, Giacherio DA, et al. Serum percent free PSA does not predict extraprostatic spread of prostate cancer. *Am J Clin Pathol* 1998; 5: 533-9.
3. France MW, Seneviratne CJ. Screening for prostate cancer: case finding is not the problem. *Ann Clin Biochem* 1997; 34: 333-8.
4. Armbruster DA. Prostate specific antigen: biochemistry, analytical methods and clinical application. *Clin Chem* 1993; 39: 181-95.
5. Bunting PS. A guide to the interpretation of serum prostate specific antigen levels. *Clin Biochem* 1995; 28: 221-41.
6. Stamey TA, Yang N, Hay AR, et al. Prostate specific antigen as a serum marker for adenocarcinoma of prostate. *N Eng J Med* 1987; 317: 909-16.
7. Lilja H, Christensson A, Dahlen U, et al. Prostate specific antigen in serum occurs predominantly in complexes with a 1 antichymotrypsin. *Clin Chem* 1991; 37: 1618-25.
8. Pettersson K, Piironen T, Seppala M, et al. Free and complexed prostate specific antigen (PSA): in vitro stability, epitope map and development of immunofluorometric assays for specific sensitive detection of free PSA and PSA α -1 antichymotrypsin complex. *Clin Chem* 1995;41:1480-6.
9. Oesterling JE, Jacobson SJ, Klee GG, et al. Free, complexed and total serum prostate specific antigen: the establishment of appropriate reference ranges for their concentrations and ratios. *J Urol* 1995;154:1090-5.
10. Partin AW, Oesterling JE. The clinical usefulness of prostate specific antigen: update. *J Urol* 1994;152:1358-68.
11. Duffy MJ. PSA as a marker for prostate cancer: a critical review. *Ann Clin Biochem* 1996;33:511-19.
12. Schreiber WE. Free prostate specific antigen: does it measure up? editorial. *Am J Clin Pathol* 1998;5:511-13.
13. Dew T, Coker C, Saadeh F, Mulvin D, Coptcoat MJ, Sherwood RA. Influence of investigative and operative procedures on serum prostate specific antigen concentration. *Ann Clin Biochem* 1999; 36: 340-6.
14. Woodrum DL, Brawer MK, Partin AW, et al. Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J Urol* 1998; 159: 5-12.
15. Christensson A, Bjork T, Nilsson O, et al. Serum prostate specific antigen complexed to α 1antichymotrypsin as an indicator of prostate cancer. *J Urol* 1993;150:100-5.
16. Catalona WJ, Smith DS, Wolfert RL, et al. Evaluation of percentage of free serum PSA to improve specificity of prostate cancer screening. *JAMA* 1995; 274: 1214-20.
17. Vashi AR, Wojno KJ, Henrick BA, et al. Determination of reflex range and appropriate cut points for percent free prostate specific antigen in 413 men referred for prostatic evaluation using the AxSYM system. *Urology* 1997; 49: 19-27.
18. Lauderer A, 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: 187-94.
19. Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: A prospective multicenter clinical trial. *JAMA* 1998; 279: 1542-7.
20. Price CP, Allard J, Davies G, et al. Pre and post analytical factors that may influence use of serum prostate specific antigen and its isoforms in a screening programme for prostate cancer. *Ann Clin Biochem* 2001; 38: 188-216.
21. Brawer MK, Duam P, Petteway JC, Wener MH. Assay variability in serum prostate specific antigen determination. *Prostate* 1995; 26: 1-6.

