Original Article

Evaluation of diagnostic accuracy of different biomarkers for prostate cancer

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ABSTRACT

Objective: Serum total prostate specific antigen (PSA), free fraction of total prostate specific antigen percent (fPSA %) and prostate specific antigen density (PSAD) have all been considered as valuable non-invasive tumor markers for prostate cancer. This study was carried out to evaluate and compare the performances of serum total PSA, fPSA% and PSAD in terms of their sensitivity, specificity and overall diagnostic accuracy for prostate cancer.

Methodology: Fifty (50) DRE (digital rectal examination)-positive patients admitted in Rajshahi Medical College Hospital (RMCH), Bangladesh during January, 2006 to January, 2008 were included. Estimation of serum total PSA and fPSA% were done by ELISA (Enzyme linked immunosorbent assay) using commercially available kits. Data pertaining to volume of prostate as determined by transabdominal ultrasonography were used to calculate PSAD and histology of the surgically resected prostatic tissue was done for laboratory confirmation of prostate cancer for all patients. Diagnostic sensitivity, specificity and accuracy of serum total PSA, fPSA% and PSAD were calculated using standard formulae against histopathological diagnosis.

Results: Prostate cancer was revealed in 41 of 50 patients by histopathological examination with mean age of 71.2 ±10.1 years. There were 9 cases detected as Nodular Hyperplasia of Prostate (NHP) with prostate-specific biomarkers mostly within their normal range. The sensitivity, specificity and overall diagnostic accuracy for prostate cancer of serum total PSA (at cut off value of $\geq$10 ng/ml) were 80.48%, 88.90% and 82.00%, for serum fPSA (at cut off value of $\leq$25%), were 92.68%, 77.80% and 90.00% and for PSAD (at a cut off value of > 0.15 ng/ml/cm^3), were found to be 90.00%, 88.90% and 90.00% respectively. Histologically, 27 (65.85%), 13 (31.71%) and 01 (2.44%) cases were labeled as poorly diffentiated, moderately differentiated and well differentiated carcinoma respectively and overwhelming majority had excellent correlation with all prostate-specific biomarkers.

Conclusion: These results reinforce that different prostate-specific biomarkers have good diagnostic prediction with free PSA percent and PSAD have slightly better diagnostic accuracy over serum total PSA for prostate cancer.

KEY WORDS: Prostate cancer, PSA, Free PSA percent, PSAD, Sensitivity, Specificity, Diagnostic accuracy.

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INTRODUCTION

Prostate cancer is the most common form of noncutaneous malignancy in men and its incidence varies among population of different geographic areas.[1,2] For more than a half century, serum acid phosphatase had been considered as the gold standard for the diagnosis of prostate cancer.[3] However, in 1979, Wang and his colleagues isolated

prostate-specific antigen (PSA) from prostate tissue and since then it has revolutionized the early non-invasive diagnosis as well as its role in the assessment of therapeutic response of prostate cancer.

Since its discovery, estimation of PSA has been considered as a very important tumor marker for prostate cancer. In general, PSA level below 4 ng/ml has a low probability of having clinically detectable prostate cancer but its level above 4 ng/ml is considered abnormal. PSA level of 4-10 ng/ml is a diagnostic gray zone and >10 ng/ml strongly suggests prostate cancer.[4] But as a diagnostic dilemma, PSA level <4 ng/ml has also been found among prostate cancer patients and elevated level i.e., >4 ng/ml is also associated with many benign prostatic conditions especially with nodular hyperplasia of prostate (NHP), which is a common occurrence in men over 50 years.[5] Therefore, attempts have been made to improve its diagnostic specificity including age adjustment, gland volume adjustment and serial measurement. Many investigations have suggested that estimation of unbound or free fraction of prostate specific antigen (free PSA/total PSA X 100) is an improvement of original PSA level and considered as better non-invasive diagnostic tool for prostatic cancers.[6] The most commonly proposed role of free PSA (fPSA) is an adjunct to PSA in the so called diagnostic gray zone of PSA.[7] Free PSA percent is lower in prostate cancer than other benign prostatic conditions and fPSA of >25% indicate lower risk of cancer[8] than its level of ≤25%. Further, prostate-specific antigen density (PSAD), the ratio of the serum to the size of the prostate calculated as total serum PSA divided by prostate volume has also been correlated as an improvement to total PSA for the prostate cancer. PSAD at a cut off point of 0.15 ng/ml/cm^3 has been found significantly more specific with a higher positive predictive value than other tests and a higher PSAD indicates greater likelihood of prostate cancer.[9]

Considering the importance of different biomarkers for the early diagnosis as well as for adopting better treatment strategy for prostate cancer, the present study was undertaken to evaluate and compare the diagnostic sensitivity, specificity and accuracy of serum total PSA, free PSA percent and PSAD among suspected prostate cancer cases. To the best of our knowledge, this is going to be the first published report on prostate tumour markers from Rajshahi Medical College Hospital, a 550 bed tertiary care hospital in the Northern part of Bangladesh.

METHODOLOGY

Patients: Fifty (50) clinically suspected and DRE-positive patients aged ≥50 years those who were admitted in Rajshahi Medical College Hospital (RMCH), Bangladesh during January, 2006 to January, 2008 were included for this prospective study.

Sample collection: After obtaining informed written consent, a single sample of 3.0 ml venous blood was collected following aseptic technique by venipuncture before DRE. The serum (about 1.0 ml) was collected after centrifugation of blood sample at 4000 rpm for 10 minutes and preserved into a 1.5 ml microcentrifuge tube for estimation of both total and free PSA by ELISA.

Laboratory procedures: ELISA for total PSA test (Catalog No. EIA-1778, Lot No. RN-31167, QC Reference No. 13912, DRG International, USA): The ELISA for estimation of PSA was performed by Ex 808 Multiskan ELISA reader for all 50 cases. The assay system utilized 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) was added as conjugate. The standard procedure as per manufacturer's instructions for ELISA was followed.

ELISA for free PSA test (Catalog No. EIA-1792, Lot No. RN-30943, QC Reference No. 13827, DRG International, USA): The f-PSA ELISA test was a solid phase two-site (sandwiched) immunoassay. An anti-f-PSA monoclonal antibody was coated on the surface of the microtiter wells and a rabbit anti-PSA antibody labeled with horseradish peroxidase was used as the tracer. Following standard procedure and manufacturer's instructions all the steps of ELISA were performed.

Calculation of Prostate specific antigen density: Prostate volume (length x width x anterior posterior diameter x 0.52) was measured by transabdominal ultrasonography and PSAD was calculated as follows;

$$\text{PSAD} = \frac{\text{Serum total PSA}}{\text{Prostate volume}}$$

Histopathological examination: Resected prostatic tissue from all cases was preserved in 10% buffered formalin. 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 at the Department of Pathology, Rajshahi Medical College and histological findings were noted.

Statistical analysis: 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. *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 frequency distribution of prostate cancer among different age groups . The mean age of the patients was 71.2 ± 10.1 years, with age ranging of 50-95 years. It is evident that majority of patients (42%) were in 71 – 80 years age group with 100% prostate cancer. Out of 50 clinically suspected cases of prostate cancers, 15 had their serum total PSA level in the diagnostic gray zone of 4-10 ng/ml, of which 8 were detected as carcinoma and 7 as Nodular Hyperplasia of Prostate (NHP).

Histopathological examination revealed 41 of 50 cases as prostate cancer and remaining 9 were NHP. Correlation of serum total PSA, free PSA and PSAD with frequency of prostate cancer is shown in Table-II. Out of 50 clinically suspected cases of prostate cancer, 34 (68%) had total PSA of >10 ng/ml, 40 (80%) had free PSA ≤25% and 38 (76%) had PSAD of >0.15 with frequency of cancer in 97.00%, 95% and 97.36% respectively. While among 9 cases of NHP, 7 had their percent free PSA >25%, 8 had PSAD <0.15 ng/mL/cm^3 but 7 cases were in the diagnostic gray zone for total PSA.

According to Gleason's score, number of poorly diffentiated, moderately differentiated and well differentiated carcinoma cases were 27 (65.85%), 13 (31.71% and 01 (2.44%) respectively and overwhelming majority had excellent correlation with all prostate-specific biomarkers.

Table-II: Detection rate of prostate cancer by different tumor markers (n = 50).

Tumor Markers	*No. of cases*	*No. of cancer*
Total serum PSA (Cut-off value ≥ 10 ng/ml)	34 (68.00)	33 (97.00)
Free PSA (Cut-off value ≤25%)	40 (80.00)	38 (95.00)
PSAD (Cut-off value > 0.15 ng/mL/cm^3)	38 (76.00)	37 (97.36)

(Figures in the parenthesis indicate percentage)

Table-I: Frequency distribution of prostate cancer among different age groups of patients (n = 50)

Age (years)	*No. of patients*	*Frequency of prostate cancer*
Up to 60	03 (06.00)	03 (100)
61 – 70	15 (30.00)	07 (46.66)
71 – 80	21 (42.00)	21 (100)

(Figures in parenthesis indicate percentage)

The sensitivity, specificity and over all diagnostic accuracy of serum total PSA, free PSA and PSAD are summarized in Table-III. It is evident from the table that, the sensitivity, specificity and over all diagnostic accuracy of all biomarkers are good with free PSA and PSAD were found to be slightly better than serum total PSA.

DISCUSSION

Until recently, screening for prostate cancer consisted primarily of a DRE for men over 50 years of age as part of their regular annual physical checkup. But studies have shown that use of PSA (total and/or free) in combination with rectal examination is a more sensitive method for early detection of prostate cancer.[10] After 50, age-specific incidence of prostate cancer increases three to four folds for every 10 year increase in age[11] and we also noted the increasing trend of cancer after 5th decade among the study population (Table-I).

Our attempt to evaluate diagnostic sensitivity, specificity and accuracy of different prostate specific biomarkers has revealed all to be suitable for the purpose, however, free PSA was found to be a better tumor marker over total PSA in terms of sensitivity and accuracy (92.68% vs 80.48% and 90% vs 82%). These results are in accordance with others.[7,8,12] It was noted that 95.00% patients with prostate cancer had free PSA of ≤25%, while only 30.00% had fPSA of >25%. Although different cut-off values for free PSA ranging from <10 to 25% have been used by different authors with varying but acceptable sensitivity and specificity but maximum cases (around 95%)

Table-III: Sensitivity, specificity and diagnostic accuracy of serum total PSA, free PSA percent and PSAD for prostate cancer.

Tumor markers	*Sensitivity*	*Specificity*	*Diagnostic accuracy*
Serum total PSA	80.48%	88.90%	82.00%
Free PSA percent	92.68%	77.80%	90.00%
PSAD	90.00%,	88.90%	90.00%

prostate cancer was found to be associated with ≤25% cut-off value in different studies and our findings are well consistent with them.[13] In practice, estimation of free PSA percent has been claimed to be superior over total PSA to minimize the problem of diagnostic gray zone, i.e., PSA level between 4 to 10 ng/ml and we also noted the superiority of fPSA percent over total PSA among the diagnostic gray zone cases. Further, as far as the role of PSAD for prostate cancer is concerned, we found it also to be superior marker over total PSA at a cut-off value of >0.15 ng/ml/cm^3. In the present study, 97.36% prostate cancer was associated with PSAD >0.15 (Table II), which is consistent with several investigations showed that PSAD is significantly higher in prostate cancer compared to NHP and may be valuable aid in their differentiation.[14,15]

The sensitivity, specificity and diagnostic accuracy observed for different biochemical parameters in the present study (Table-III) are in accordance with findings of others.[7,12] Although the diagnostic accuracy for each of these tests observed in the present study is quite encouraging, but in comparison, the diagnostic accuracy of free PSA (at a cut of value of <25%) and PSAD (at a cut of value of >0.15 ng/ml/cm^3) have been found better than total PSA specially for cases in the diagnostic gray zone of total PSA. These results suggest additional value of fPSA% beyond that provided by PSA alone, but the ultimate assessment of the significance of fPSA% for early cancer detection may only come from a prospective trial that would compare its use to alternate approaches. Until new markers with unique and independent advantage are available, it is suggested that fPSA and PSAD are better screening biomarkers than total PSA for the early diagnosis of prostate cancer if interpretations are made with caution.

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