# Potential biomarkers for differentiation of benign prostatic hyperplasia and prostate cancer

N. T. EL MELEGY*, H. A. ABOULELLA†, A. M. ABUL-FADL* and N. A. MOHAMED*

*Departments of *Biochemistry and †Urology, Faculty of Medicine, Assiut University, Assiut, Egypt*

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**ABSTRACT**

This study aims to evaluate the role of free/total prostate-specific antigen (PSA) ratio, serum total sialic acid level and cathepsin D activity in the differentiation of prostate cancer and benign prostatic hyperplasia (BPH). The study looked at 100 patients with BPH, 75 patients with organ-confined or locally advanced prostate cancer, and a control group of 50 healthy volunteers. Prostate cancer patients showed significantly higher total sialic acid level and cathepsin D activity and lower free/total PSA ratio than those in the BPH group. The results suggest that combined measurement of serum total sialic acid and/or cathepsin D activity with free/total PSA ratio could serve as a useful adjunct to conventional diagnostics for the differentiation of prostate cancer and BPH.

KEY WORDS: Cathepsin D.
N-Acetylneuraminic acid.
Prostate-specific antigen.
Prostatic hyperplasia.
Prostatic neoplasms.

## Introduction

Prostate cancer is the fourth most common malignant neoplasm in men worldwide and the second most common cause of cancer death.[1] Despite the recent advances in imaging modality, it remains difficult to diagnose organ-confined disease, as 25–50% of patients with clinically organ-confined disease show extraprostatic spread on histopathology.[2]

Prostate-specific antigen (PSA) is a serine protease, the gene for which is part of the human tissue kallikrein gene family. In the 1990s the discovery of PSA revolutionised early prostate cancer detection. Since then, PSA has become an important marker for diagnosis and follow up of prostate cancer. However, despite its remarkable performance, PSA is not specific for cancer.

Prostate-specific antigen sensitivity and specificity are too low to make it the ideal screening test for prostate cancer, as increased levels can occur in prostatitis and benign prostatic hyperplasia (BPH), resulting in significant numbers of false-positive cases. Hence, there is a need for new markers that better differentiate benign and malignant lesions.[3]

To improve the specificity of total PSA (tPSA), several approaches based on PSA derivatives have been investigated. These include age-specific values, PSA density (PSAD), PSAD of the transition zone, PSA velocity and assessment of various PSA isoforms.[4] Prostate-specific antigen exists in several forms, the majority of which bind to protease inhibitors (mostly α1-antichymotrypsin [ACT]) and are known as complexed PSA (cPSA). Approximately 5–35% of tPSA is not bound and is known as free PSA (fPSA). The ratio of fPSA to tPSA (f/tPSA) has been used to increase specificity for prostate cancer and to reduce unnecessary biopsies. The proportion of PSA complexed to ACT is higher and the percentage fPSA is correspondingly lower in patients with prostate cancer.[5]

With recent advances in biotechnology, many potential blood biomarkers have been identified and are currently under investigation.[3] Many emerging markers that show some promise for prostate cancer diagnosis are at various stages of development. These include human glandular kallikrein, early prostate cancer antigens, insulin-like growth factor-I (IGF-I) and its binding proteins (IGFBP-2 and IGFBP-3), urokinase plasminogen activation system, transforming growth factor-β1, interleukin-6, chromogranin A, prostate secretory protein, prostate-specific membrane antigen, hepsin, prostate cancer-specific autoantibodies and α-methylacyl-CoA racemase. While these and other markers have shown promise in early-phase studies, no single biomarker is likely to provide the appropriate degree of certainty to dictate treatment decisions.[4] Given the plethora of possible biomarkers available, this study evaluates the potential blood-based biomarkers sialic acid and cathepsin D.

Sialic acid (N-acetylneuraminic acid) is a negatively-charged monosaccharide derivative, attached by an α-glycosidic linkage to the non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids. Prostate cancer cells have more sialic acid in their cell membrane than either normal or BPH cells.[6]

Cathepsin D is a lysosomal cysteine protease involved in lysosomal protein degradation. The high expression levels of serum cathepsin D in prostatic intraepithelial neoplasia and invasive adenocarcinoma of the prostate suggests that it might play an important role in early prostate cancer changes.[7]

The future of cancer prognosis may rely on small panels of markers that can accurately predict prostate cancer presence, stage and metastasis, and serve as indicators, targets and/or surrogate end points of disease progression and response to therapy.[4] Therefore, this study aims to evaluate the role of

*Correspondence to: Dr Nagla T. El Melegy*
*Biochemistry Department, Faculty of Medicine, Assiut University, Assiut, Egypt*
*Email: elmelegynagla@yahoo.com*

f/t PSA ratio, serum total sialic acid level and cathepsin D activity in distinguishing BPH and prostate cancer in urological referral patients.

## Materials and methods

One hundred seventy-five consecutive male patients were enrolled in the study. They were admitted to the urology department, Assiut University Hospital, from May 2004 to November 2005. All referred patients had lower urinary tract symptoms. The patients were classified in two groups. The first included 100 patients with histopathologically proved BPH (age range: 50–78 years [mean±SD: 68.95±8.64]). The second group included 75 patients with histopathologically confirmed prostate cancer. All studied prostate cancer patients had organ-confined or locally advanced adenocarcinoma of the prostate (stages T1c, T2 and T3) (age range: 52–79 years [mean±SD: 66.6±7.29]).

A control group of 50 healthy male volunteers was included in the study (age range: 45–68 years [mean±SD: 57.72+6.11]). This group had no lower urinary tract symptoms and the prostate was normal by digital rectal examination (DRE) and transrectal ultrasound examination (TRUS).

All patients gave a full history, underwent thorough clinical examination and TRUS examination of the prostate. A TRUS-guided biopsy was performed on the lesion or taken from six sites in those without a definite lesion and those with BPH. Final diagnosis was confirmed histopathologically by examining tissue obtained by transurethral resection of the prostate (TURP) or by open transvesical prostatectomy for prostate weighting >80 g.

Patients suspected clinically of having prostate cancer were given a chest X-ray and a radioisotope bone scan to exclude osseous metastasis. Patients were excluded from the study if urine culture was positive, there was a history of acute urinary retention or indwelling urethral catheter, or a history of cystoscopy or TRUS needle-guided biopsy within the previous three months, or there was evidence of malignancy elsewhere or other chronic disease (cardiac, hepatic or renal), or total PSA <20 ng/mL.

Informed consent was obtained from all participants and the study was approved by the Ethical Committee of Faculty of Medicine, Assiut University.

Venous blood (5 mL) was obtained from each patient and control. The samples were allowed to clot at room temperature, and then centrifuged at 4000 x*g* for 15 min. Serum was divided in aliquots and stored at –20°C until assay for fPSA, tPSA, serum total sialic acid (TSA) and cathepsin D activity.

Serum fPSA was determined by an enzyme-linked immunosorbent assay (ELISA) kit (BC-1021, Biocheck, Canada) according to the method of Vashi *et al*.[8] Serum tPSA was determined by an ELISA kit (BS-1102, Biosewoom, Korea) according to the method described by Kuriyama *et al*.[9] The f/t PSA ratio was calculated for each case. Serum TSA was determined by the method of Plucinsky *et al*.[10] Serum cathepsin D activity was determined by a modification of the haemoglobin method described by Kuhn and Kock.[11]

The results were analysed using SPSS and Prism programs, and the results presented as mean±SD. $P<0.05$ was considered statistically significant. Data comparisons were performed using two-tailed, unpaired Student's *t*-test and the Mann-Whitney test.

**Table 1.** Serum levels of fPSA, tPSA and fPSA:tPSA ratio in patients with BPH, prostate cancer and controls.

| | Controls ($n$=50) | BPH ($n$=100) | Prostate cancer ($n$=75) |
|---|---|---|---|
| fPSA (ng/mL) | | | |
| Mean±SD | 0.21±0.13 | 5.04±2.98 | 4.18±2.88 |
| Range | 0.10–0.60 | 0.30–10.87 | 1.20–11.19 |
| *P* value | | *<0.001 | *<0.001 †NS |
| tPSA (ng/mL) | | | |
| Mean±SD | 0.20±0.09 | 8.95±5.98 | 12.21±6.07 |
| Range | 0.10–0.38 | 1.26–19.60 | 2.56-19.85 |
| *P* value | | *<0.001 | *<0.001 †<0.05 |
| fPSA:tPSA ratio | | | |
| Mean±SD | 1.39±1.04 | 0.65±0.31 | 0.36±0.18 |
| Range | 0.31–6.00 | 0.10–1.27 | 0.09-0.77 |
| *P* value | | *<0.01 | *<0.001 †<0.001 |

SD: Standard deviation.

*Compared with controls.

†Compared with BPH group.

NS: Not significant.

## Results

In the BPH group, 14 patients (14%) had a tPSA <4 ng/mL, 40 patients (40%) had tPSA of 4–10 ng/mL and 46 patients (46%) had tPSA >10 ng/mL. In the prostate cancer group, 11 patients (14.7%) had a tPSA <4 ng/mL, 26 patients (34.6%) had tPSA of 4–10 ng/mL and 38 patients (50.7%) had tPSA >10 ng/mL. Serum fPSA, tPSA and f/t PSA in patients with BPH, prostate cancer and in the controls are shown in Table 1. It shows significantly higher mean fPSA and tPSA, and significantly lower f/t PSA in patients with BPH and prostate cancer in comparison to the control group ($P<0.001$). Patients with prostate cancer showed significantly lower f/t PSA in comparison to those with BPH ($P<0.001$).

Serum TSA and cathepsin D in patients with BPH and prostate cancer, and in the control group are shown in Table 2. Mean serum TSA was significantly higher in prostate cancer patients compared to those with BPH ($P<0.001$) and the control group ($P<0.001$). Patients with prostate cancer showed significantly higher mean cathepsin D activity compared to those with BPH and the control group ($P<0.001$). No correlation was found between the studied parameters.

## Discussion

Different concepts have been used to enhance the specificity of PSA measurement without losing sensitivity, including PSA velocity, density and age-specific reference ranges. Different molecular forms of PSA exist in serum, of which the most important are fPSA, PSA complexed to α1-antichymotrypsin (PSA-ACT) and PSA complexed to α2-macroglobulin.[5]

The present study reveals significantly higher mean serum tPSA in patients with BPH and prostate cancer compared

with controls. No significant difference was observed when comparing tPSA in prostate cancer patients to those with BPH. This finding was consistent with previous findings that tPSA is not an ideal tumour marker as it is organ-specific rather than carcinoma-specific. Furthermore, the value of tPSA for early detection of prostate cancer is controversial because there is an appreciable false-positive rate, resulting in many unnecessary biopsies.[12] Also, not all patients with prostate cancer have an elevated tPSA: 30–40% of men with organ-confined prostate cancer have a normal PSA level.[13] These data are consistent with the findings of the present study.

As the biology of PSA isoforms is poorly understood and the change in PSA isoforms is an early event, it would be a manifestation of a premalignant field change. Furthermore, it has been reported that f/t PSA ratio improves early detection of prostate cancer and decreases the number of biopsies, and could be an early sign of increased prostate cancer risk.[14]

In the present study, patients with prostate cancer had non-significant lower mean fPSA compared to those with BPH. This agrees with the findings of Espana *et al*.[15] About 10–20% of tPSA circulates as a free form in blood. The decrease in fPSA levels observed in prostate cancer patients could be attributed to the higher production of ACT by tumour cells. This may enhance the formation of the complex between PSA and ACT and subsequently decrease serum fPSA.[15]

The present study revealed significantly lower f/t PSA ratios in prostate cancer patients compared to those in BPH patients. These results agree with the findings of other studies.[1,16–18] However, Masters *et al*. reported that the usefulness of the f/t PSA ratio in the assessment of patients with suspected prostate cancer is not established and does not appear to be sufficiently accurate to be used alone as a predictor of cancer.[19] Moreover, the study by Lee *et al*. proposed that f/t PSA ratio can help to determine which men with a PSA of 4–10 ng/mL should have a biopsy; however, the %fPSA appears to be most useful at extreme ratios.[20] Also, de la Taille *et al*. found that f/t PSA might prevent 21–31% of unnecessary biopsies. This demonstrates the great variability in the recommended threshold values and in the reference ranges among studies. One possible explanation for the different reported thresholds is that the measurements were made under different analytical conditions.[21]

As sialic acid is a major constituent of glycoproteins and glycolipids, several investigators have studied levels in sera or plasma of patients with different malignant diseases. Most of these have looked at total sialic acid and/or glycolipid-bound sialic acid. The former includes glycoprotein- and glycolipid-bound sialic acid as well as a small amount of free sialic acid, whereas the later includes only glycolipid-bound sialic acid which is not a cancer-specific marker.[22]

In the present study, significantly elevated serum TSA was seen in patients with prostate cancer compared to BPH patients and the control group. These findings are in agreement with previous study.[22] Hobarth *et al*. reported that the assessment of sialic acid in patients with BPH or prostatitis yields a high proportion of false-positive results, but the rate is markedly lower in patients with prostate cancer. They also reported higher TSA in patients with distant metastasis than in those with organ-confined disease.[23] Also, a direct relationship has been shown between elevated serum sialic acid and tumour progression.[6]

**Table 2.** Serum levels of total sialic acid and cathepsin D activity in patients with BPH, prostate cancer and controls.

| | | Controls ($n$=50) | BPH ($n$=100) | Prostate cancer ($n$=75) |
|---|---|---|---|---|
| Total sialic acid (mg/dL) | Mean±SD | 6.25±3.49 | 7.24±4.56 | 15. 1±4.83 |
| | Range | 1.0–13.2 | 1.1–17.6 | 8.4-29.2 |
| | *P* value | | *NS | *<0.001 †<0.001 |
| Cathepsin D activity (pmol/mL) | Mean±SD | 4.85±1.35 | 5.11±1.17 | 9.2±1.75 |
| | Range | 2.5–7.0 | 2.5–7.8 | 6.8–12.8 |
| | *P* value | | *NS | *<0.001 †<0.001 |

SD: Standard deviation.
*Compared with controls.
†Compared with BPH group.
NS: Not significant.

Moreover, a marked drop in sialic acid was noted in prostate cancer patients who responded to chemotherapy.[24] However, Romppanen *et al*.[25] reported that a good response to treatment in patients with prostate cancer was reflected in a statistically significant reduction in PSA but not in TSA. These findings agree with the present observation of no correlation between PSA and TSA.

As serum sialic acid lacks tumour specificity, it is not helpful in screening for prostate cancer, yet it might contribute towards the early detection of tumour progression and metastasis during therapy and follow-up.[23] Clearly, further studies of serum sialic acid during therapy and follow-up are necessary to determine its relevance as a tumour marker in patients with prostate cancer.

Cathepsin D is one of the most important enzymes in invasion and metastatic dissemination, and increased production is seen in different malignant tissues.[26] In the present study, mean serum cathepsin D was significantly higher in patients with prostate cancer than in those with BPH and in controls. These findings are in agreement with the work of Cherry *et al*., who reported that both normal and BPH specimens predominantly express procathepsin D, while significantly greater expression of cathepsin D was noted in prostate cancer patients. They suggest that an increase in cathepsin D may be a prerequisite for progression and/or metastasis in prostate cancer.[7] Miyake *et al*. reported that using systematic biopsy with serum cathepsin D and/or PSA could predict the incidence of extraprostatic disease.[27]

The high level of cathepsin D observed in prostate cancer patients could be attributed to the fact that activation of cathepsin D requires an acidic microenvironment which is present in prostatic tumour tissue due to increased production of lactic acid via anaerobic glycolysis. Lactic acid is capable of activating cathepsin D, thus the development of an acidic microenvironment could provide favourable conditions for the activation of cathepsin D *in vivo*, which may lead to tumour progression and metastasis.[28]

The present study also showed no correlation between serum cathepsin D and PSA, which agrees with the study of Gómez Díaz *et al*.[29] and Hara *et al*.[30] They attributed this finding to the small number of tumour samples studied and to the short follow-up. Also, Cherry *et al*.[7] reported that PSA

was at least one of the physiological substrates for cathepsin D in the prostate, suggesting that cathepsin D might modulate PSA action in the appropriate microenvironment. Therefore, serum cathepsin D activity could be regarded as a clinical factor independent of serum PSA. Measurement of serum cathepsin D could serve as a useful practical adjunct to conventional diagnostic tools for the differentiation of prostate cancer and BPH. Clearly, further long-term studies correlating cathepsin D with tumour progression and metastasis are warranted.

In conclusion, f/t PSA ratio enhances the ability to discriminate between patients with prostate cancer and BPH. The combined measurement of TSA and f/t PSA ratio might contribute towards early tumour detection. Moreover, measurement of serum cathepsin D could serve as a useful adjunct in the conventional diagnosis of prostate cancer. These findings warrant further investigation on a larger population to improve the clinical use of these biomarkers for discriminating patients with an early, potentially curable prostate cancer and those with BPH. □

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