Complexed and total PSA in patients with benign prostatic hyperplasia and prostate cancer

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ABSTRACT

This study compares the diagnostic utility of complexed prostate-specific antigen (cPSA), total PSA (tPSA) and their ratios with free PSA (fPSA) for benign prostatic hyperplasia (BPH) and prostate cancer. This is though to be the first study to evaluate cPSA in the ethnic population of Saudi Arabia. Serum samples were collected from 54 patients (aged over 50) and assayed for tPSA, cPSA and fPSA. Thirty-five patients were histologically and clinically proven to have BPH and 19 patients were proven to have cancer. Sensitivity, specificity and ROC curves were calculated. With a cPSA cut-off of 4 ng/mL the sensitivity was 79%, the specificity was 34%, and the positive and negative predictive values (PPV and NPV) were 39% and 75%, respectively. At the same cut-off for tPSA, the sensitivity was 84%, the specificity was 29%, and the PPV and NPV were 39 and 77%, respectively. The sensitivity for both tests was lower at a cut-off of 20 ng/mL but the specificity increased to 77% for cPSA and 69% for tPSA. The areas under the receiver operating characteristic (ROC) curves were found to be 0.608 for tPSA and 0.559 for cPSA ($P=0.69$). The incidence of prostate cancer in the Saudi population may be lower than that in Western populations. The data presented show little advantage in using cPSA over tPSA for discriminating BPH and prostate cancer in the population studied.

KEY WORDS: Prostatic hyperplasia.
Prostatic neoplasms.
Prostate-specific antigen.

Introduction

Prostate-specific antigen (PSA) is a single-chain glycoprotein polypeptide consisting of 237 amino acid residues.[1] The biological function of PSA is believed to be liquefaction of the seminal clot formed in freshly ejaculated semen,[2] which aids sperm motility and may have a role in fertility.

Improvements in cancer detection using PSA have come from an understanding of the various molecular forms of PSA in serum. It was initially assumed that PSA measured in serum by immunoassays was the natural form of the 33 kDa protein, but it was later discovered that PSA in serum is largely co-valently attached to the serum protease inhibitor α1-antichymotrypsin (ACT) and that only a relatively small portion of serum PSA is present in non-complexed form.[3] Thus, the terms 'free' PSA (fPSA) and 'complexed' PSA (cPSA) are used to distinguish non-complexed from complexed PSA.

Immunoassays were developed to distinguish free PSA and total PSA (tPSA; free plus complexed PSA) and it was discovered that a lower ratio of fPSA correlates with a higher risk of prostate cancer.[4] In contrast, a higher percentage of fPSA correlates with benign disease such as benign prostatic hyperplasia (BPH). Many different PSA antibodies have been developed to distinguish fPSA from cPSA and tPSA.[5] However, despite the success of fPSA in cancer detection, limitations remain and better serum markers are needed. As fPSA is the serum component that provides better cancer discrimination, investigations have focused on further discriminating fPSA into different molecular forms. This is because fPSA represents the inactive form that cannot complex with ACT.

Recent studies support the hypothesis that fPSA contains different molecular forms of inactive PSA, and that these forms correlate with specific aspects of prostate disease.[6] Conversely, cPSA is relatively homogeneous in serum and is present in only trace amounts in benign and malignant prostate tissues.[7] Therefore, the study of cPSA appears to offer no inherent cancer specificity.

The ratio of fPSA to tPSA (fPSA:tPSA) is used to differentiate prostate cancer from BPH, with semi-elevated levels of tPSA 4–10 ng/mL.[8] Studies have shown that cPSA is a useful prognostic tool; however, its sensitivity is lower than obtained with the fPSA:tPSA ratio.[9]

In current medical practice, serum PSA values >10 ng/mL indicate >50% chance of cancer.[10] Values in the range (4–10 ng/mL) suggest the need for prostate biopsy. However, only 20–40% of men with levels in this range will be found to have prostate cancer.[11] Traditionally, men with PSA values <4 ng/mL have not been biopsied but in recent years it has been shown that 20–30% of men with serum PSA in the range 2–4 ng/mL will have prostate cancer.[12] Therefore, the current PSA range of interest for the early detection of cancer is 2–10 ng/mL, with growing emphasis on better cancer detection in the lower ranges (2–6 ng/mL).

This study aims to compare the diagnostic utility of cPSA,

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tPSA and their ratios with free PSA (fPSA) for benign prostatic hyperplasia (BPH) and prostate cancer in an ethnic population of Saudi Arabia.

Materials and methods

This study was performed in King Fahad National Guard Hospital (KFNGH) in Riyadh. Blood samples were collected in serum separator tubes (Greiner Bio-One, Germany) and centrifuged for 10 min at 3000 rpm. Serum samples were separated and either assayed immediately or stored at –70°C (not for more than one month due to sample instability of PSA *in vitro*). Three levels of quality control materials were used (Bio-Rad, USA) for each assay.

A total of 4773 patients were screened for tPSA during 2006. The majority (93%) were aged over 50 years (range: 43–98 [average: 65]). When tPSA was <4 ng/mL or >10 ng/mL no further testing was performed. cPSA levels were determined for any patients with a tPSA value in the range 4–10 ng/mL. cPSA levels were also measured for any clinically suspected case, regardless of the tPSA value. Serum tPSA and cPSA were measured by immunoassay (Advia Centaur; Siemens, USA). The %fPSA values were then calculated from the previous two assays based on the formula tPSA=fPSA+cPSA.[13] Serum fPSA and %fPSA levels were measured by an immunoassay method (Immulite immunoassay analyser; Siemens, USA).

Prostate biopsy and other tests were performed only when the cases were clinically suspected and when PSA was in the range 4–10 ng/mL. When prostate cancer was diagnosed then the staging of the disease was defined by the Whitmore-Jewett system (A and B: cancer confined to the prostate; C: local spread but not to lymph nodes; D: metastasis to lymph nodes or elsewhere).

In the cPSA assay (Advia Centaur), fPSA is prevented from reacting with tPSA antibodies by incubating the sample with a fPSA-specific monoclonal mouse antibody (petreatment reagent). The assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses equal amounts of two antibodies. The first is a polyclonal goat anti-PSA antibody labelled with acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-PSA antibody that is co-valently coupled to paramagnetic particles. The analytical sensitivity values of tPSA and cPSA are 0.01 and 0.03 ng/mL, respectively.

The Immulite method is a chemiluminometric immunoassay. The sensitivity and specificity at different cut-off values (4 and 20 ng/mL) were measured and, where possible, the receiver operating characteristic (ROC) curves and area under the curve (AUC) were calculated and compared. In addition, as a part of the evaluation and validation process, the Advia Centaur assay was compared to the Immulite assay.

The two-tailed P values were calculated using the GraphPad Software (www.graphpad.com). $P \leq 0.05$ was considered statistically significant.

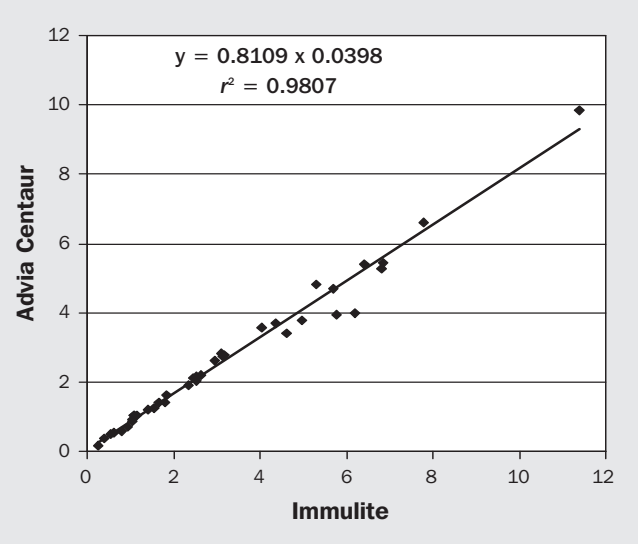

Fig. 1. Correlation between tPSA (Immulite) and tPSA (Advia Centaur) in Saudi benign prostatic hyperplasia (BPH) and prostate cancer (PCa) patients. Both are chemiluminescence immunoassays.

Results

A total of 54 (1.13%) patients were confirmed to have abnormal tPSA and/or cPSA values. When further clinical, radiological and/or histological testing was performed, 35 (0.74%) were confirmed with BPH and 19 (0.40%) with prostate cancer. Table 1 shows the distribution of cPSA and tPSA values <4.0, 4–10, 10–20 and >20.0 ng/mL among patients with BPH (n=35) and cancer (n=19).

Figure 1 shows the correlation between the Immulite and Advia Centaur methods with respect to tPSA as calculated by linear regression analysis. The coefficient of determination (r^2) was found to be 0.98. Figure 2 shows a correlation of 0.91 between the cPSA and tPSA values, both from the Advia Centaur. Figure 3 shows the coefficient determination (r^2) for the measured %fPSA versus the calculated %fPSA to be 0.9412. Figure 4 shows the coefficient of determination (r^2) between the measured %fPSA and

Table 1. Distribution of cPSA and tPSA values among 54 patients with either benign prostatic hyperplasia (BPH) or prostate cancer (PCa). The system of Whitmore-Jewett was used to stage the patients with prostate cancer (n=19).

PCa stage (n)	Values (ng/mL)	Age range (years)	cPSA (%)		tPSA (%)	
			BPH (n=35)	PCa (n=19)	BPH (n=35)	PCa (n=19)
Stage A (3)	<4.0	43–82	3 (6%)	2 (4%)	4 (7%)	1 (2%)
Stage B (1)	4.1–10.0	69–93	4 (7%)	1 (2%)	3 (6%)	0 (0%)
Stage C (7)	10.01–20.0	55–88	10 (19%)	8 (15%)	18 (33%)	4 (7%)
Stage D (8)	>20.0	61–98	18 (33%)	8 (15%)	10 (19%)	14 (26%)

%cPSA to be 0.91. At the cut-off value of 4 ng/mL for cPSA the sensitivity was 79%, specificity was 34%, and the positive and negative predictive values (PPV and NPV) were 39% and 75%, respectively (Table 1). At the same cut-off for tPSA the sensitivity was 84%, specificity was 29%, and PPV and NPV were 39% and 77%, respectively. At the cut-off value of 20 ng/mL the sensitivity for both tests was reduced to 51% and 53%, respectively, but specificity increased to 77% and 69%, respectively. *P* values were 0.56 and 0.69, respectively. Areas under the ROC curves were 0.608 for tPSA and 0.559 for cPSA (P=0.69).

Discussion

The incidence of benign and malignant prostate conditions in the hospitalised Saudi population is low compared to Western populations.[14] This is consistent with previous reports which indicate that prostate cancer is rare in Asian populations.[15] Other studies reported a rate of 2.7% among Saudis, but these data are now 15 years old.[16,17] This is higher than the level found here; however, neither the present study nor other local studies have examined an adequate sample size.

According to the national cancer registry, prostate cancer is the fifth leading cancer (5.9%) among Saudi men affected by the disease.[18] In another epidemiological study, Ezzat *et al*. retrospectively evaluated a total of 22,836 different types of cancer cases in Saudi Arabia between 1976 and 1993.[19] They reported that prostate cancer was not in the top 10 most commonly encountered cancers in any age group or region presented.[19] Ranking adult (male and female) cancer cases by system revealed that there were 1192 (5.2%) cases of cancer of the urinary system (ranking ninth) and 568 (2.5%) cases of cancer of the male reproductive system (ranking 12th). The rate in their study was higher than report here, but still lower than reports of Western populations.

Another study performed by one of the authors reviewed the records of 16,617 adult males over the age of 45 years between 1984 and 1996. This found that 74 (0.44%) patients were diagnosed with prostate malignancy.[20] It also found that the mean age of the presented cases was 76.8 years, and 20 patients were younger than 65 years of age.

Sehai reported that more than 70% of prostate cancer patients were admitted to hospitals with advanced-stage disease.[21] This study estimated the incidence of prostate cancer to be around 800 new cases per million population per year.[21] Another study reported poor knowledge about prostate cancer and high levels of misperception in this region.[22]

Two-thirds of our cases were benign, which is consistent with results reported by Mansoor *et al*.[17] Froehner *et al*. compared the clinical value of the measurement of cPSA and tPSA to discriminate BPH and prostate cancer.[23] They concluded that there was a statistically significant advantage to the use of cPSA compared with tPSA in this regard. However, the difference was small and its clinical relevance is questionable.[23] The present study found that sensitivity of cPSA and tPSA was high; however, specificity at different cut-offs was low.[24] Brawer *et al*. reported that cPSA provided an 11% improvement in specificity over tPSA, resulting in fewer false-positive cases.[25]

Data on 356 biopsy-confirmed patients showed that utilising a cut-off of 3.6 ng/mL for cPSA provided a 38.7% PPV.[26] In an evaluation of cPSA in 137 patients with tPSA of 4–10 ng/mL, researchers from Japan concluded that tPSA might be replaced by cPSA in the diagnosis of prostate cancer. This study proved that in the range 4–10 ng/mL cPSA was better able to predict prostate biopsy outcome than a commercially available tPSA assay.[27]

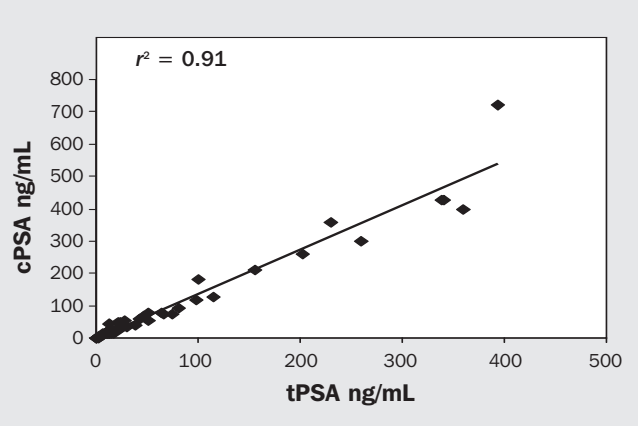

Fig. 2. Correlation between complexed (cPSA) and total (tPSA) values from the Advia Centaur analyser.

In a multi-centre study of 439 men with tPSA values of 4–10 ng/mL, it was shown that cPSA was able to reduce the number of false-positive results. Based on the improvement in specificity, the authors concluded that cPSA could serve as a single assay replacement for the measurement of tPSA.[25] In another study, of 130 men with tPSA values in the range 4–10 ng/mL, a similar level of improvement was observed with cPSA.[28] The results presented here do not suggest any advantage of using cPSA over tPSA. Specificity improved when these tests were combined with other histological, radiological and clinical findings (data not shown) but not as one independent marker to differentiate benign from malignant prostate disease.

Many of the assays used in the studies referred to above have been improved (especially tPSA) and therefore comparison of the utility of testing for different forms of PSA may need to be re-evaluated. Furthermore, although specificity has improved, it is still relatively low. This was reported earlier by Jung *et al*., who used the same technique as that employed in the present study. They found that fPSA:tPSA and cPSA:tPSA ratios improve the differentiation between BPH and cancer and are effective in reducing the rate of unnecessary biopsies, whereas cPSA alone does not.[29]

The differences between the results of many studies of tPSA and between tPSA and various other forms of PSA may be attributed to the fact that PSA assays historically have produced different results. Clearly, therefore, better

Table 2. Sensitivity and specificity of tPSA and cPSA at cut-off values of 4 ng/mL and 20 ng/mL.

Test	Cut-off	Sensitivity	Specificity
tPSA	4	84%	29%
cPSA	4	79%	34%
tPSA	20	53%	69%
cPSA	20	51%	77%

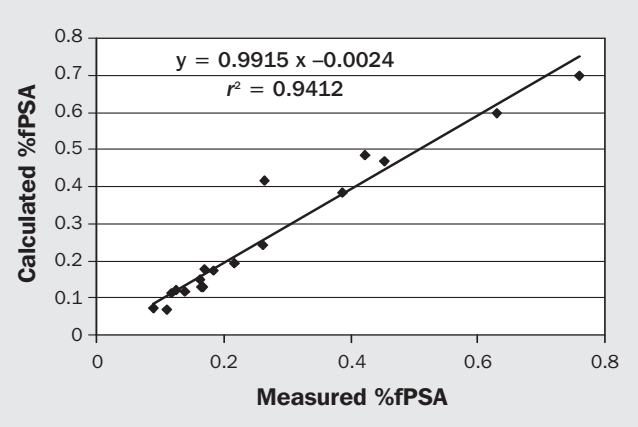

Fig. 3. Correlation between measured %fPSA (Immulite) and calculated %fPSA.

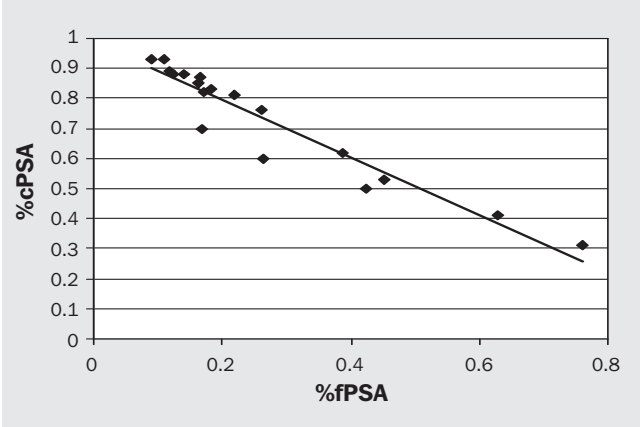

Fig. 4. Correlation between measured %fPSA (Immulite) and measured %cPSA (Advia Centaur).

harmonisation of PSA values between the different PSA test systems must be realised.[30] However, differences among tPSA assays appear to have decreased since the introduction of the World Health Organization (WHO) 96/670 reference preparation, but further efforts are needed to harmonise fPSA and cPSA assays.[31]

These discrepancies may also be attributed to underlying disease. For example, haemodialysis procedures induce elevation in PSA forms, but tPSA is the least affected. cPSA has not shown any diagnostic superiority over other PSA forms, and thus serum tPSA remains a reliable parameter for follow-up of prostate cancer in uraemic patients on long-term dialysis.[32]

In order to improve screening for prostate cancer and to decrease disease-specific mortality, new prostate biomarkers should be evaluated. Raaijmakers *et al*. evaluated human kalikrin 2 (hK2) and %fPSA and found that they added prognostic value for the detection of minimal prostate cancer in screen-detected cases within the PSA range 4–10 ng/mL.[33] Shariat *et al*. reported an increase in plasma urokinase-type plasminogen activator (uPA) and its soluble receptor (uPAR) in patients with prostate cancer[34] Another promising marker, early prostate cancer antigen (EPCA)-2, was proposed by Leman *et al*. and is associated with prostate cancer, has higher sensitivity and specificity than PSA and accurately differentiates men with organ-confined and non-organ-confined disease.[35] Zhang *et al*. developed a method to detect prostate cancer micrometastasis by a quantitative real-time polymerase chain reaction (RT-PCR) method. They found a significant difference in the PSA and prostate-specific membrane antigen (PSMA) messenger mRNA levels among BPH, locally confined cancer and metastatic disease in blood samples.[36]

Hirano *et al.* showed that plasma chromogranin A (CgA) levels in prostate cancer increase with the severity of the disease, especially in progressive hormone-refractory prostate cancer (HRPC), after hormone therapy. Although this cross-sectional study involved only a small number of patients, they believe that plasma CgA levels may predict HRPC status and prognosis in metastatic disease.[37]

In conclusion, it appears that the rate of prostate cancer in Saudi Arabia is low but the importance of screening protocols and increasing education and awareness cannot be over-emphasised, and further large-scale screening studies in this population are recommended. Few new markers are available to improve screening for prostate cancer, and this is another area in which further research is required. □

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