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Utilizing Prostate Specific Antigen Density to Enhance Diagnostic Accuracy and Minimize Unnecessary Biopsies

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Abstract

To evaluate the effectiveness of PSA density in distinguishing between benign prostatic hyperplasia (BPH) and prostate cancer in patients with intermediate PSA levels (4 - 10 ng/ml) and to reduce unnecessary biopsies. **Patients and Methods:** 90 patients with PSA levels in the "Gray Zone" PSA (4 - 10 ng/ml) were included. Prostate volumes were estimated using transrectal ultrasonography. Serum PSA levels were measured using an enzyme immunoassay. PSA density was calculated and compared with histopathological results. Statistical analysis was performed using SPSS, t-test, and Contingency Coefficient test. **Results:** A PSA density cutoff of 0.15 ng/ml/cm^3 was used for prostate cancer detection. Mean PSA density for BPH patients: 0.0844 ng/ml/cm^3. Mean PSA density for prostate cancer patients: 0.172 ng/ml/cm^3. Sensitivity: 92.3%, Specificity: 93.5%, Positive Predictive Value: 70.5%, Accuracy: 93.3%. PSA density significantly reduced unnecessary biopsies ($p < 0.001$). **Conclusions:** PSA density can be effective in reducing unnecessary biopsies in patients with intermediate PSA levels, particularly those with organ-confined prostate cancer who are candidates for radical treatment.

Keywords

PSA Density, Benign Prostatic Hyperplasia (BPH), Prostate Cancer, Unnecessary Biopsies, Intermediate PSA Levels

1. Introduction

Prostate cancer (CaP) is the most common visceral malignant neoplasm in men

and the second leading cause of cancer-related deaths in men [1]. Worldwide prostate cancer incidence and mortality rates vary significantly between countries and regions and are highest in African American men [2]. It is predominantly a disease of the elderly and is diagnosed below the age of 50 in less than 0.1% of all patients. Peak incidence occurs between the ages of 70 and 74 years. Latent cancers of the prostate are commonly observed in autopsy [3]. Histologically, over 95% of the cancers of the prostate are adenocarcinomas. Of the remaining 5%, transitional cell carcinomas account for 95% and the remaining cancers are neuroendocrine ("small cell") carcinomas or sarcomas [4]. Prostate carcinomas are often asymptomatic, especially in their early stages. Around 20% of localized prostate cancers are incidentally detected during histological examinations of prostate tissue removed for suspected benign hyperplasia. Autopsy studies have shown that the incidence can reach up to 50% in African American men over 70 years old. [5] Since most prostate cancers originate in the peripheral regions of the prostate, they may be found during routine digital rectal exams.

In more advanced stages, or when the tumors are larger, they may cause lower urinary tract symptoms (LUTS), such as local discomfort and urinary obstruction, similar to benign prostatic hyperplasia. On physical examination, these cases often present with a hard, immobile prostate, suggesting locally advanced disease. Sometimes, prostate carcinomas are first identified through symptoms of metastasis, with bone metastases—particularly in the axial skeleton—being common. These metastases may be either osteolytic (destructive) or, more frequently, osteoblastic (bone-forming).

The presence of osteoblastic metastases is strongly suggestive of advanced prostatic carcinoma [6].

A commonly used tool for grading prostate carcinoma is the Gleason system. Scores from 6 to 10 are graded into 5 grade groups, which are further used to define the risk groups of prostate cancer.

Clinically, CaP has symptoms like benign prostate hypertrophy (BPH), which is also the most common benign pathology in men. The incidence of BPH is age-related and autopsy studies have revealed that the prevalence of histologic BPH ranges from approximately 20% in men aged 41 - 50; to 50% in men aged 51 - 60; and to over 90% in men older than 80 [7].

Prostate-specific antigen (PSA) is a 33-kilodalton glycoprotein that functions as a serine protease [8]. Although it was first identified and purified in the late 1970s, its widespread application in clinical urology did not occur until the subsequent decade. Clinically, PSA is considered organ-specific, being predominantly produced by prostatic luminal epithelial cells [9]. Under normal conditions, PSA is present in the serum at low concentrations, measured in nanograms per milliliter.

In the serum, PSA circulates in both bound and unbound forms. The majority of serum PSA is bound or complexed with antiproteases such as alpha-1-antichymotrypsin (ACT) and macroglobulin [10]. Conversely, free PSA lacking proteolytic activity is likely rendered inactive within prostatic epithelial cells before being

released into the serum. This inactive, unbound-free PSA does not form complexes with antiproteases, circulates freely, and is detectable immunologically using current assays [11].

Age-specific reference ranges for PSA levels have been established: 0 to 2.5 ng/mL for individuals aged 40 - 49 years, 0 to 3.5 ng/mL for those aged 50 - 59 years, 0 to 4.5 ng/mL for ages 60 - 69 years, and 0 to 6.5 ng/mL for individuals aged 70 - 79 years [12]. Following the complete removal of prostate tissue, the serum half-life of PSA has been calculated to be approximately 2 to 3 days [13].

The presence of prostate diseases such as prostate cancer, benign prostatic hyperplasia (BPH), and prostatitis are the most significant factors influencing serum PSA levels. While elevated PSA levels may indicate the presence of prostate disease, not all men with such conditions exhibit increased PSA levels. Moreover, PSA elevations are not exclusively indicative of prostate cancer [14]. PSA serves as a sensitive yet non-specific screening tool because both benign and malignant conditions can lead to elevated serum PSA levels. In prostate cancer, elevated serum PSA levels are likely due to cancer progression and the disruption of the prostate's histological architecture. Patients with prostatitis or benign prostatic hyperplasia often present with elevated PSA levels; however, in these cases, evaluations for prostate cancer may ultimately be unnecessary [12].

Early studies established a reference range of 0 to 4.0 ng/mL to define normal serum PSA levels. This range was determined using the Tandem-R PSA assay (Hybritech, San Diego, CA) in a cohort of 37 healthy men, demonstrating that all healthy men under 40 years of age and 97% of men over 40 years had PSA levels equal to or less than 4.0 ng/mL. These findings were confirmed in subsequent studies [15].

The choice of a PSA threshold or cut-off point above which one would recommend further evaluation to rule out prostate cancer (prostate biopsy) is different among guidelines [16] [17]. In fact, there is still uncertainty as to whether PSA levels can be used as a specific marker for diagnosing CaP [18]. As serum PSA levels increase with age, age-specific ranges have been defined in older men to increase the detection of significant but potentially curable tumors per specific age group. Furthermore, while the PSA threshold of 4 ng/mL is commonly used, the use of single cut off PSA value for all men may risk the exclusion of an unacceptably high number of patients with clinically significant early-stage prostate cancer [16]. At cut off level of 4 ng/mL, the PSA value has a sensitivity of about 20% and a specificity of about 93.6% [19]. Men with prostate-specific antigen (PSA) levels exceeding 10 ng/mL and a benign digital rectal examination (DRE) have up to a 60% likelihood of being diagnosed with prostate cancer. These individuals are unlikely to benefit from further enhancements in PSA sensitivity and specificity. The percentages become even more significant for PSA levels greater than 20 ng/mL or when the DRE is suspicious for prostate cancer; following surgery in these cases, many cancers are found to have extended beyond the prostate gland. Therefore, men with PSA levels above 10 ng/mL are encouraged to undergo a biopsy [20].

For men whose PSA levels fall between 4 and 10 ng/mL, achieving specificity in cancer detection is more challenging. Cancers identified within this range are often at earlier stages and potentially more curable but may also represent "insignificant," potentially non-life-threatening tumors [15]. Due to the considerable overlap in serum PSA concentrations between men with and without prostate cancer, this range is referred to as the diagnostic "grey zone." Efforts to improve diagnostic accuracy within this grey zone include the utilization of PSA density (PSAD), PSA velocity, and age-specific PSA levels (**Table 1**). Although the efficacy of these PSA derivatives in enhancing the specificity of total PSA is debated among physicians, they have become additional tools for assessing prostate cancer risk during clinical consultations [21].

Table 1. Prostate-specific antigen derivatives.

PSA Derivative	Definition	Threshold Values	Limitations
PSA density	Serum PSA/prostate volume	≥0.15 associated with the presence of prostate cancer	-Variations in prostate size, shape -ratio of stromal to epithelial tissue -Variations in ultrasound/MRI measurement
PSA velocity	Rate of change of PSA	>0.75 ng/mL per year	-Variations in assay -lack of previous results -Need for extended wait to make clinical recommendations
Age-specific PSA	Age-specific normalized PSA values		-Risk missing significant cancer in older men -overdetection in younger men

A direct correlation between PSAD and the likelihood of cancer has been documented [22]. In men with a PSA between 4.0 and 10.0 ng/mL and a normal DRE, a PSAD greater than 0.15 is considered suspicious for the presence of cancer [23]. However, the usefulness of PSAD in prostate cancer detection has not been confirmed in all studies. For example, Brawer and colleagues found that PSAD did not improve the predictive ability of PSA levels alone in detecting cancer among men with PSA values of 4 to 10 ng/mL and normal DRE findings [24]. Conversely, several other studies have confirmed its usefulness in prostate cancer screening [25]. Higher PSA densities are often observed in groups of men with positive biopsy results compared to those with negative biopsies. This is because prostate cancers are more likely to be detected when a constant number of biopsies are performed in men with smaller prostate volumes than in those with larger volumes [26]. Variations in the amount of epithelial tissue—the source of PSA—between prostates of similar size may also play a role.

Currently, no noninvasive technique exists to precisely quantify the epithelial contribution to total PSA levels. Additionally, the variability in prostate morphology

poses challenges for the consistent application of a standardized volume equation to estimate prostate size.

To understand the sensitivity and specificity of PSAD in predicting early-stage CaP, we undertook a prospective study enrolling patients in Iraq. Problems with PSA test alone and overlapping PSA between BPH and CaP (in the grey zone levels) were the main reasons to undertake this study. We also aimed to study if this would help in diagnosis of early-stage CaP and decrease rate of unnecessary biopsy.

2. Aim of the Study

The aim of this study was to assess the additional value of prostate-specific antigen density (PSAD) as diagnostic marker to differentiate patients with benign prostatic hyperplasia from patients with cancer of the prostate in 'grey zone' PSA level and if there could be any reduction in the need for unnecessary biopsies in this patient population.

3. Patients and Methods

This study was performed in the Department of Urology; Rizgary Teaching Hospital in Erbil, Iraq, between September 2017 and January 2019. Ninety patients aged 55 - 89 years with clinically confined prostatic disease who underwent surgery for prostate were assessed by determination of total serum PSA and transrectal ultrasound (TRUS) examination and were enrolled into the study after giving informed consent.

Data collection began after obtaining approval from the Kurdistan Ministry of Health's Ethical Committee in August 2017 using questionnaire forms and specific data points included patient's age, date of admission, laboratory investigations (PSA, complete blood count, erythrocyte sedimentation rate, blood urea, serum creatinine, blood group, Rh type, general urine examination), radiological imaging and their findings (TRUS, abdominal kidney ultrasound, and CT scan of pelvis when indicated), preoperative prostatic biopsy in selected cases, type of surgery done for the patient and postoperative prostatic histopathological examination for all patients.

To maintain consistency, all the surgeries were performed by the same surgeon. The type of surgery performed in these 90 patients was either transurethral resection of the prostate (TURP) in 62 patients, retropubic prostatectomy (26 patients), or radical prostatectomy (2 patients). TRUS examinations were performed using a SONAOACE$_{X4}$ version 2.00.00 scanners with a 6.5 MHz probe (Multiplane 3-D) and were carried out by the same radiologist who had experience in performing TRUS. Prostatic volume was calculated by ellipsoid formula, which requires the measurement of 3 prostate dimensions. The ellipsoid volume formula is applied as follows: Volume = height × width × length × 0.52 [27].

Serum total PSA was measured in the same laboratory by ELISA method using monoclonal antibody test. PSA density was calculated as PSA density = serum PSA/prostate gland volume (PSAD = PSA/V).

The histopathological examination of the surgically removed prostate was performed by the same pathologist. The validity of PSAD (*i.e.* sensitivity, specificity, positive predictive value) was assessed using the histopathological results as gold standard.

A standard statistical software program (SPSS 17.0 for Windows) was used to analyze the data. t-test was used to determine the difference between variables, while Contingency Coefficient test was used to relate between variables. A $p < 0.05$ was significant, and $p \leq 0.001$ was regarded to be highly significant.

4. Results

The mean age of the studied sample was 67.9 years (range 55 - 90 ± SD 6.22), in the benign group the mean age was 67.7 years (range 56 - 80 years ±SD 9.08) and in the malignant group 69 years (range 60 - 89 years ± SD 5.66). The age distribution of patients is shown in Table 2.

Histopathological examination of surgically removed prostate revealed prostate cancer in 13 patients and benign pathology in 77 patients in Table 3.

Table 2. Distribution of patients according to their age.

AGE (years)	Benign		Malignant		Total	
	N	(%)	N	(%)	N	(%)
55 - 59	6	(7.7)	1	(7.7)	7	(7.8)
60 - 69	40	(53)	6	(46.2)	46	(51)
70 - 79	30	(38.1)	5	(38.4)	35	(40)
80 - 89	1	(1.2)	1	(7.7)	2	(2.2)
Total	77	(100.0)	13	(100.0)	90	(100.0)

Table 3. The results of histopathological examination.

HISTOPATHOLOGY	N	(%)
Malignant	13	(14.4)
Benign	77	(85.6)
Total	90	(100.0)

All patients with benign group had normal DRE. DRE for the malignant group was normal in 38.4% of cases and abnormal (nodular or hard) prostate in 61.6% of cases with a sensitivity of 61.53 in detecting CaP as shown in Table 4.

All patients with the benign group had normal TRUS finding apart from calcification in some patients. While TRUS for the malignant group was normal in 30.76% of cases and abnormal (hyperechoic, or heterogeneous) in 69.24% of cases, with a sensitivity of 69.2 in detecting CaP as shown in Table 5.

In our study, there was no correlation between PSAD and Gleason score obtained from surgical specimens as shown in Table 6. The mean PSA of the studied

Table 4. Distribution of patients according to TRUS findings.

TRUS	Benign		Malignant		Total	
	N	(%)	N	(%)	N	(%)
Normal	77	(100)	4	(30.8)	81	(90)
Abnormal	0	0	9	(69.2)	9	(10)
Total	77	(100.0)	13	(100.0)	90	(100.0)

Table 5. PSAD, Gleason score in malignant group.

N	PSAD (ng/mL^2)	Gleason score
1	0.12	7 (4 + 3)
2	0.154	6 (3 + 3)
3	0.16	7 (4 + 3)
4	0.164	8 (5 + 3)
5	0.17	6 (3 + 3)
6	0.17	PIN
7	0.177	5 (2 + 3)
8	0.178	7 (3 + 4)
9	0.2	7 (3 + 4)
10	0.2	PIN
11	0.204	7 (3 + 4)
12	0.213	3 (1 + 2)
13	0.215	3 (1 + 2)

PIN: Prostate Intraepithelial Neoplasia.

Table 6. Distribution of patients according to PSA value.

PSA (ng/mL)	Benign		Malignant		Total	
	N	(%)	N	%	N	%
4 - 4.9	23	(29.9)	-	-	23	(25.6)
5 - 5.9	27	(35)	1	(7.7)	28	(31.1)
6 - 6.9	12	(15.6)	2	(15.4)	14	(15.6)
7 - 7.9	3	(3.9)	2	(15.4)	5	(5.6)
8 - 8.9	3	(3.9)	3	(23.1)	6	(6.7)
9 - 9.9	9	(11.7)	5	(38.4)	14	(15.6)
Total	77	(100.0)	13	(100.0)	90	(100.0)

sample was 6.23 ng/mL (range 4 - 9.8 ng/mL ± SD 1.79). In the benign group the mean PSA was 5.905 ng/mL (range 4 - 9.8 ng/mL ± SD 1.638) and in the malignant

group 8.197 ng/mL (range 5.5 - 9.8 ng/mL ± SD 1.361). The PSA distribution of patients is shown in Table 7.

The mean volume of prostate in the studied sample was 75.94 mL (range 23.4 - 190 mL ± SD 36.9). In the benign group the mean volume was 80.545 mL (range 23.4 - 190 ml ± SD 37.681) and in the malignant group 48.123 mL (range 31 - 63 mL ±SD 10.604). The type of surgery performed for the patients is shown in Table 8 and the volume distribution of patients is shown in Table 9.

Table 7. Distribution of patients according to prostate volume. In patients with prostate volume more than 100 mL, elevation of PSA can be caused by increase in prostate size rather than cancer.

Prostate volume (mL)	Benign		Malignant		Total	
	N	(%)	N	(%)	N	(%)
23 - 39	5	(6.5)	-	-	5	(5.55)
40 - 59	22	(28.5)	3	(23.1)	25	(27.77)
60 - 79	21	(27.3)	7	(53.8)	28	(31.11)
80 - 99	12	(15.6)	3	(23.1)	15	(16.66)
100 - 119	4	(5.2)	-	-	4	(4.4)
120 - 139	5	(6.5)	-	-	5	(5.6)
140 - 159	2	(2.6)	-	-	2	(2.2)
160 - 179	5	(6.5)	-	-	5	(5.6)
180 - 190	1	(1.3)	-	-	1	(1.1)
Total	77	(100.0)	13	(100.0)	90	(100.0)

Table 8. Distribution of patients according to PSAD.

PSAD (ng/mL^2)	Benign		Malignant		Total	
	N	(%)	N	(%)	N	(%)
>0.15	5	(6.5)	12	(92.3)	17	(18.9)
<0.15	72	(93.5)	1	(7.7)	73	(81.1)
Total	77	(100.0)	13	(100.0)	90	(100.0)

Table 9. Correlation between PSAD and histopathology.

PSAD (ng/mL^2)	HISTOPATHOLOGY					
	Benign		Malignant		Total	
	N	(%)	N	(%)	N	(%)
>0.15	5	(6.5)	12	(92.3)	17	(18.9)
<0.15	72	(93.5)	1	(7.6)	73	(81.1)
Total	77	(100.0)	13	(100.0)	90	(100.0)

The mean PSAD was 0.0844 (range 0.03 - 0.252 ± SD 0.038) in benign group and 0.172 (range 0.12 - 0.213 ± SD 0.025) in malignant group. PSAD distribution is shown in Table 8.

Correlation of histopathology with age, PSA, prostate volume, and PSAD is shown in Table 2, Table 7, Table 8, Table 9.

Histopathological findings in relation to PSAD are shown in Table 9, the correlation between histopathology and PSAD was highly significant, $p < 0.001$.

This study shows sensitivity of 92.3%, and specificity of 93.5% when PSAD is added to PSA in comparison to PSA alone.

5. Discussion

The detection of early prostate cancer depends on the accuracy of DRE, TRUS and serum PSA, which are currently considered to be the best tools for the diagnosis of this disease [17]. In the PSA range there is a considerable overlap in serum PSA concentrations in men with and without prostate cancer. This range has been described as the diagnostic "grey zone". PSAD has been considered to be a better tool in differentiating between patients with BPH and prostate cancer [28] [29]. The primary goal of utilizing PSA density (PSAD) is to identify clinically significant prostate cancers, either by recommending immediate biopsy for patients with a PSAD > 0.150 or by considering delayed biopsy based on additional indicators, such as elevated PSA velocity.

While not exposing the entire population (benign) to biopsy and not allowing patients with significant cancers to experience a significant delay in diagnosis. The advantage of PSAD over PSA velocity is that PSAD is immediate while PSAV results can be interpreted only after a minimum of a 12 - 18 months follow-up, there is no clear-cut evidence to support the use of PSAV as a diagnostic predictive tool to indicate the need for biopsy or in active surveillance [30].

By relating serum PSA values to the volume of the prostate gland, using PSAD, Benson *et al.* [31] [32] demonstrated an effective method of reducing the number of false-positive PSA values. PSAD is based on the premise that, under normal circumstances each epithelial cell (producing PSA) will require a given amount of stromal support (prostate volume) to maintain normal structure and function. While normal prostate tissues and BPH may adhere to this rule, CaP does not. Furthermore, BPH grows by expansion, whereas CaP grows by expansion and infiltration, which will affect cell number (PSA production) without altering prostate volume [33].

The aim of this prospective analysis was to investigate whether PSAD-based results could help in the diagnosis of prostate cancer and assist in avoiding a significant number of biopsies. This study is the first study performed in Iraq population.

In this study, we observed an overlap in PSA value between benign and malignant prostate (5.905 ng/mL vs 8.197 ng/mL). In patients with BPH (77/90) 85.6% the mean PSAD was 0.0844 ng/mL/cm^3 and only five patients (5/77) 6.49% had

false positive result with PSAD more than 0.15. The result of histopathology in those five patients showed moderate to extensive invasion of polymorphic white blood cells and the biopsies were histologically classified as benign prostatic nodular hyperplasia, glandular and fibromuscular with chronic non-specific prostatitis.

In the subset of patients with prostate cancer (13/90) 14.4% the mean PSAD was 0.172 ng/mL^2 and only one patient (1/13) 7.69% had false negative result with PSAD less than 0.15. In this patient, DRE was negative with TRUS showing single hyperechoic lesion with massive calcification.

If a PSA cutoff of 4 ng/mL is used for the detection of CaP, 85.55% of patients in the grey zone will undergo unnecessary prostate biopsy. On the other hand, PSAD when used as an additional test for those patients with PSA values in the gray zone (iPSA) 5.55% of patients will undergo unnecessary biopsy, while in 1.11% of the patients the diagnosis of CaP would be missed.

The correlation between the results of histopathology and the PSAD was highly significant, $p < 0.001$, and PSAD with a cutoff of 0.15 ng/mL^2 showed sensitivity of 92.3%, a specificity of 93.5%, with a positive predictive value of 70.5 % and accuracy of 93.3%.

We must realize that PSAD is an additional tool in CaP detection. Its usefulness has not been confirmed in all studies. Catalona and colleagues found that PSAD failed to detect up to 50% of cancers in men with PSA values within the diagnostic gray zone [34]. Another study which was performed by Wolff *et al* where PSAD limit of 0.15 was used and showed a sensitivity of 77.3%, a specificity of 80.0% and a positive predictive value of 81.0% [35]. Van Iersel in their study in on 91 patients, showed that in BPH patients mean PSA was 6.8 ng/mL, while in CaP it was 7.5 ng/mL; in BPH the mean volume was 50.0 mL and in CaP patients it was 40.0 mL in CaP and the mean PSAD of 0.13 ng/mL^2 in BPH and 0.19 ng/mL^2 in CaP with $p = 0.027$ [36]. In our study, the mean PSA was 5.90 ng/mL in BPH and 8.19 ng/mL in CaP, the mean volume of 80.54 mL in BPH and 48.12 in CaP, mean PSAD 0.08 in BPH and 0.172 in CAP with $p < 0.001$. The conflicting results with PSAD could be caused by variations in the amount of epithelium (source of PSA) between prostates of similar size, but there is no noninvasive method for determining how much epithelium is contributing to overall PSA. It can also be caused by variability in prostate shape limiting the use of a common volume equation for calculating prostate size.

The reliability of PSAD is a product of the reliability of its constituents. The accuracy of PSAD obviously depends on the accuracy with which the PSA and prostatic volume are assessed. The production of PSA per volume of prostatic tissue is not only related to the presence of BPH and prostate cancer but also to the proportion of epithelial cells and to the histological grade of the carcinoma [37].

In our study we found that there is no correlation between PSAD and Gleason score and we had no possibility to calculate the proportion of epithelial cells (producing PSA?) in pathology slides.

Certain limitations need to be noted in our study. Prostatic inflammation (acute

and chronic) and urinary retention can cause PSA elevations to variable degrees [38]. Prostatic trauma such as occurs after prostatic biopsy can result in a leak of PSA into the circulation that may require more than 4 weeks for return to baseline values [39]. DRE as performed in an outpatient setting can lead to increases in serum PSA [40] [41]. However, the change in PSA after DRE would not appear to be clinically significant. Ejacualtion can lead to an increase in PSA within 48 hours, and this fractional increase in PSA is expected to return to baseline levels in most men. A history of sexual activity and a repeated PSA test after 48 hours of sexual abstinence may be helpful in the interpretation of serum PSA levels that are minimally elevated [42]. Other limitations include a small cohort of patients studied and intra-person variability arising due to the sonologist performing TRUS.

In the future, accuracy of CaP detection can be further enhanced by using serum free-PSA density [43], MRI-based PSA density [44], or application of different cutoff value. Ongoing studies assess PSAD predictive value in certain patient populations, and integration of PSAD into decision-making for prostate biopsy which may facilitate improved risk-adjusted care.

In summary, in this study we see that PSAD could be helpful and supplementary to DRE and TRUS in detection of CaP. PSAD can be helpful in differentiating serum PSA elevations secondary to BPH from those secondary to CaP and provide additional information regarding the need for biopsy in the intermediate range between 4 and 10 ng/mL, especially in patients with organ-confined CaP who are candidates for radical treatment. PSA-based screening assessments remain the most important method of prostate cancer detection [45]· As for patients who present with the "grey zone" PSA in the range of 4 to 10 ng/mL, present a risk of performing too many unnecessary biopsies, wherein these additional tests can be of valuable support in the diagnostic process. This study confirms usefulness of PSAD in Iraq population adding important evidence to prostate cancer detection in this patient population.

Further studies including larger cohort of patients are recommended to further confirm the benefit of addition of PSAD to 'gray zone' PSA levels.

6. Additional Information

Authorship: All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work, and have given their approval for this version to be published.

Authors' contributions: Fahmi Sabr Raza, Sharadchandra K. Prasad, Sourabh Karna, Nahin M. Hoq, Yousef Abu Osba, Areen Ibrahim Saber, Mostafa Gado and Feroz Ali Khan conceptualized the manuscript and wrote the first draft.

All authors provided significant intellectual input and reviewed, edited, and approved the final manuscript.

Compliance with ethics guidelines: This article is based on a study that has received appropriate ethical and institutional approvals.

Consent to publish: All authors have reviewed the final version of this manuscript and provided their consent to publish.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Jemal, A., Tiwari, R.C., Murray, T., Ghafoor, A., Samuels, A., Ward, E., *et al.* (2004) Cancer Statistics, 2004. *CA*: *A Cancer Journal for Clinicians*, **54**, 8-29. https://doi.org/10.3322/canjclin.54.1.8

[2] Hinata, N. and Fujisawa, M. (2022) Racial Differences in Prostate Cancer Characteristics and Cancer-Specific Mortality: An Overview. *The World Journal of Men's Health*, **40**, 217-227. https://doi.org/10.5534/wjmh.210070

[3] Kimura, T., Sato, S., Takahashi, H. and Egawa, S. (2021) Global Trends of Latent Prostate Cancer in Autopsy Studies. *Cancers*, **13**, Article 359. https://doi.org/10.3390/cancers13020359

[4] Cooperberg, M.R., Presti, Jr, J.C., Shinohara, K. and Carroll, P.R. (2008) Neoplasm of the Prostate Gland. In: McAninch, J.W. and Lue, T.F., Eds., *Smith's General Urology*, McGraw-Hill Companies, 348-350.

[5] Jahn, J.L., Giovannucci, E.L. and Stampfer, M.J. (2015) The High Prevalence of Undiagnosed Prostate Cancer at Autopsy: Implications for Epidemiology and Treatment of Prostate Cancer in the Prostate-Specific Antigen-Era. *International Journal of Cancer*, **137**, 2795-2802. https://doi.org/10.1002/ijc.29408

[6] Michell, R.N. (2007) Robbins Basic Pathology. 7th edition. Elsevier.

[7] Egan, K.B. (2016) The Epidemiology of Benign Prostatic Hyperplasia Associated with Lower Urinary Tract Symptoms: Prevalence and Incident Rates. *Urologic Clinics of North America*, **43**, 289-297. https://doi.org/10.1016/j.ucl.2016.04.001

[8] Yu, H., Diamandis, E.P., Levesque, M., Sismondi, P., Zola, P. and Katsaros, D. (1994) Ectopic Production of Prostate Specific Antigen by a Breast Tumor Metastatic to the Ovary. *Journal of Clinical Laboratory Analysis*, **8**, 251-253. https://doi.org/10.1002/jcla.1860080412

[9] Wang, Z.A., Toivanen, R., Bergren, S.K., Chambon, P. and Shen, M.M. (2014) Luminal Cells Are Favored as the Cell of Origin for Prostate Cancer. *Cell Reports*, **8**, 1339-1346. https://doi.org/10.1016/j.celrep.2014.08.002

[10] Lein, M., Lein, M., Jung, K., Jung, K., Elgeti, U., Elgeti, U., *et al.* (2001) Comparison of the Clinical Validity of Free Prostate-specific Antigen, Alpha-1 Antichymotrypsin-bound Prostate-Specific Antigen and Complexed Prostate-Specific Antigen in Prostate Cancer Diagnosis. *European Urology*, **39**, 57-64. https://doi.org/10.1159/000052413

[11] Balk, S.P., Ko, Y. and Bubley, G.J. (2003) Biology of Prostate-Specific Antigen. *Journal of Clinical Oncology*, **21**, 383-391. https://doi.org/10.1200/jco.2003.02.083

[12] Jain, M.A., Leslie, S.W. and Sapra, A. (2024) Prostate Cancer Screening. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK556081/

[13] Stephan, C., Jung, K., Brux, B., Lein, M., Sinha, P., Schnorr, D., *et al.* (2000) ACT-PSA and Complexed PSA Elimination Kinetics in Serum after Radical Retropubic Prostatectomy: Proof of New Complex Forming of PSA after Release into Circulation. *Urology*, **55**, 560-563. https://doi.org/10.1016/s0090-4295(99)00548-8

[14] Frumer, M., Aharony, S.M., Shoshany, O., Kedar, D., Baniel, J. and Golan, S. (2023) Prostate-Specific Antigen Level Association with COVID-19 Infection and Vaccination. *Clinical Genitourinary Cancer*, **21**, e405-e411. https://doi.org/10.1016/j.clgc.2023.05.001

[15] Gretzer, M.B. and Partin, A.W. (2002) PSA Levels and the Probability of Prostate Cancer on Biopsy. *European Urology Supplements*, **1**, 21-27. https://doi.org/10.1016/s1569-9056(02)00053-2

[16] Tikkinen, K.A.O., Dahm, P., Lytvyn, L., Heen, A.F., Vernooij, R.W.M., Siemieniuk, R.A.C., *et al.* (2018) Prostate Cancer Screening with Prostate-Specific Antigen (PSA) Test: A Clinical Practice Guideline. *BMJ*, **362**, k3581. https://doi.org/10.1136/bmj.k3581

[17] Wei, J.T., Barocas, D., Carlsson, S., Coakley, F., Eggener, S., Etzioni, R., *et al.* (2023) Early Detection of Prostate Cancer: AUA/SUO Guideline Part I: Prostate Cancer Screening. *Journal of Urology*, **210**, 46-53. https://doi.org/10.1097/ju.0000000000003491

[18] Georgiou, L.A. and Scarbrough, B.E. (2024) PSA Screening for Prostate Cancer in the United States: 30 Years of Controversy. *Journal of Public Health Policy*, **45**, 552-561. https://doi.org/10.1057/s41271-024-00502-4

[19] Daryanto, B., Trianto, R., Seputra, K. and Purnomo, A. (2024) Evaluation of Cutoff Point Prostate Specific Antigen (PSA) and Prostate Specific Antigen Density (PSAD) in Patients with Suspected Prostate Cancer. *Medical Archives*, **78**, 12-15. https://doi.org/10.5455/medarh.2024.78.12-15

[20] Welch, H.G., Schwartz, L.M. and Woloshin, S. (2005) Prostate-Specific Antigen Levels in the United States: Implications of Various Definitions for Abnormal. *JNCI: Journal of the National Cancer Institute*, **97**, 1132-1137. https://doi.org/10.1093/jnci/dji205

[21] Jain, S., Bhojwani, A.G. and Mellon, J.K. (2002) Improving the Utility of Prostate Specific Antigen (PSA) in the Diagnosis of Prostate Cancer: The Use of PSA Derivatives and Novel Markers. *Postgraduate Medical Journal*, **78**, 646-650. https://doi.org/10.1136/pmj.78.925.646

[22] Balasubaramaniam, V., Kailasa, A. and Donev, K. (2024) 892 Prostate Specific Antigen Density (PSAD) in Predicting Prostate Cancer: A District General Hospital Analysis. *British Journal of Surgery*, **111**, znae163.737. https://doi.org/10.1093/bjs/znae163.737

[23] Nordström, T., Akre, O., Aly, M., Grönberg, H. and Eklund, M. (2017) Prostate-Specific Antigen (PSA) Density in the Diagnostic Algorithm of Prostate Cancer. *Prostate Cancer and Prostatic Diseases*, **21**, 57-63. https://doi.org/10.1038/s41391-017-0024-7

[24] Brawer, M.K., Aramburu, E.A.G., Chen, G.L., Preston, S.D. and Ellis, W.J. (1993) The Inability of Prostate Specific Antigen Index to Enhance the Predictive Value of Prostate Specific Antigen in the Diagnosis of Prostatic Carcinoma. *Journal of Urology*, **150**, 369-373. https://doi.org/10.1016/s0022-5347(17)35485-x

[25] Yusim, I., Krenawi, M., Mazor, E., Novack, V. and Mabjeesh, N.J. (2020) The Use of Prostate Specific Antigen Density to Predict Clinically Significant Prostate Cancer. *Scientific Reports*, **10**, Article No. 20015. https://doi.org/10.1038/s41598-020-76786-9

[26] Bruno, S.M., Falagario, U.G., d'Altilia, N., Recchia, M., Mancini, V., Selvaggio, O., *et al.* (2021) PSA Density Help to Identify Patients with Elevated PSA Due to Prostate Cancer Rather than Intraprostatic Inflammation: A Prospective Single Center Study. *Frontiers in Oncology*, **11**, Article 693684. https://doi.org/10.3389/fonc.2021.693684

[27] Yanai, Y., Kosaka, T., Hongo, H., Matsumoto, K., Shinojima, T., Kikuchi, E., *et al.* (2018) Evaluation of Prostate-specific Antigen Density in the Diagnosis of Prostate Cancer Combined with Magnetic Resonance Imaging before Biopsy in Men Aged 70 years and Older with Elevated PSA. *Molecular and Clinical Oncology*, **9**, 656-660. https://doi.org/10.3892/mco.2018.1725

[28] Frisbie, J.W., Van Besien, A.J., Lee, A., Xu, L., Wang, S., Choksi, A., *et al.* (2022) PSA Density Is Complementary to Prostate MP-MRI PI-RADS Scoring System for Risk Stratification of Clinically Significant Prostate Cancer. *Prostate Cancer and Prostatic*

Diseases, **26**, 347-352. https://doi.org/10.1038/s41391-022-00549-y

[29] Xing, P., Chen, L., Yang, Q., Song, T., Ma, C., Grimm, R., *et al.* (2021) Differentiating Prostate Cancer from Benign Prostatic Hyperplasia Using Whole-Lesion Histogram and Texture Analysis of Diffusion- and T2-Weighted Imaging. *Cancer Imaging*, **21**, Article No. 54. https://doi.org/10.1186/s40644-021-00423-5

[30] Javaeed, A., Ghauri, S.K., Ibrahim, A. and Doheim, M.F. (2020) Prostate-Specific Antigen Velocity in Diagnosis and Prognosis of Prostate Cancer—A Systematic Review. *Oncology Reviews*, **14**, 64-71. https://doi.org/10.4081/oncol.2020.449

[31] Benson, M.C., Seong Whang, I., Olsson, C.A., Mcmahon, D.J. and Cooner, W.H. (1992) The Use of Prostate Specific Antigen Density to Enhance the Predictive Value of Intermediate Levels of Serum Prostate Specific Antigen. *Journal of Urology*, **147**, 817-821. https://doi.org/10.1016/s0022-5347(17)37394-9

[32] Benson, M.C., Seong Whang, I., Pantuck, A., Ring, K., Kaplan, S.A., Olsson, C.A., *et al.* (1992) Prostate Specific Antigen Density: A Means of Distinguishing Benign Prostatic Hypertrophy and Prostate Cancer. *Journal of Urology*, **147**, 815-816. https://doi.org/10.1016/s0022-5347(17)37393-7

[33] Seaman, E., Whang, M., Olsson, C.A., Katz, A., Cooner, W.H. and Benson, M.C. (1993) PSA DENSITY (PSAD): Role in Patient Evaluation and Management. *Urologic Clinics of North America*, **20**, 653-663. https://doi.org/10.1016/s0094-0143(21)00918-6

[34] Catalona, W.J. and Smith, D.S. (1994) 5-Year Tumor Recurrence Rates after Anatomical Radical Retropubic Prostatectomy for Prostate Cancer. *Journal of Urology*, **152**, 1837-1842. https://doi.org/10.1016/s0022-5347(17)32397-2

[35] Wolff, J.M., Boeckmann, W., Effert, P.J., Handt, S. and Jakse, G. (1995) Evaluation of Patients with Diseases of the Prostate Using Prostate - Specific Antigen Density. *British Journal of Urology*, **76**, 41-46. https://doi.org/10.1111/j.1464-410x.1995.tb07829.x

[36] van Iersel, M.P., Witjes, W.P., de la Rosette, J.J. and Oosterhof, G.O. (1995) Prostate - Specific Antigen Density: Correlation with Histological Diagnosis of Prostate Cancer, Benign Prostatic Hyperplasia and Prostatitis. *British Journal of Urology*, **76**, 47-53. https://doi.org/10.1111/j.1464-410x.1995.tb07830.x

[37] Holmäng, S., Lindstedt, G., Mårin, P. and Hedelin, H. (1993) Serum Concentration of Prostate-Specific Antigen in Relation to Prostate Volume in 50 Healthy Middle-Aged Men. *Scandinavian Journal of Urology and Nephrology*, **27**, 15-20. https://doi.org/10.3109/00365599309180408

[38] Kwak, C., Ku, J.H., Kim, T., Park, D.W., Choi, K.Y., Lee, E., *et al.* (2003) Effect of Subclinical Prostatic Inflammation on Serum PSA Levels in Men with Clinically Undetectable Prostate Cancer. *Urology*, **62**, 854-859. https://doi.org/10.1016/s0090-4295(03)00688-5

[39] Yuan, J.J.J., Coplen, D.E., Petros, J.A., Figenshau, R.S., Ratliff, T.L., Smith, D.S., *et al.* (1992) Effects of Rectal Examination, Prostatic Massage, Ultrasonography and Needle Biopsy on Serum Prostate Specific Antigen Levels. *Journal of Urology*, **147**, 810-814. https://doi.org/10.1016/s0022-5347(17)37392-5

[40] Figueirêdo, M.d.F., Lopes, G.T. and Naidu, T.G. (2003) Digital Rectal Examination (DRE) Does Not Influence Total Serum Levels of Prostate Specific Antigen (tPSA), in Individuals without Prostate Pathology. *International braz j urol*, **29**, 423-427. https://doi.org/10.1590/s1677-55382003000500006

[41] Chybowski, F.M., Bergstralh, E.J. and Oesterling, J.E. (1992) The Effect of Digital Rectal Examination on the Serum Prostate Specific Antigen Concentration: Results of a Randomized Study. *Journal of Urology*, **148**, 83-86. https://doi.org/10.1016/s0022-5347(17)36517-5

[42] Demir, K., Tarhan, F., Orcun, A., Aslan, H. and Turk, A. (2014) Effects of Ejaculation on Serum Prostate-Specific Antigen Levels. *Türk Üroloji Dergisi/Turkish Journal of Urology*, **40**, 40-45. https://doi.org/10.5152/tud.2014.03704

[43] Zou, B., Wen, H., Luo, H., Luo, W., Xie, Q. and Zhou, M. (2023) Value of Serum Free Prostate-Specific Antigen Density in the Diagnosis of Prostate Cancer. *Irish Journal of Medical Science* (1971-), **192**, 2681-2687. https://doi.org/10.1007/s11845-023-03448-w

[44] Aphinives, C., Nawapun, S. and Tungnithiboon, C. (2023) Diagnostic Accuracy of MRI-Based PSA Density for Detection of Prostate Cancer among the Thai Population. *African Journal of Urology*, **29**, Article No. 4. https://doi.org/10.1186/s12301-023-00335-9

[45] Bratt, O., Auvinen, A., Arnsrud Godtman, R., Hellström, M., Hugosson, J., Lilja, H., *et al.* (2023) Screening for Prostate Cancer: Evidence, Ongoing Trials, Policies and Knowledge Gaps. *BMJ Oncology*, **2**, e000039. https://doi.org/10.1136/bmjonc-2023-000039