

Prostate Cancer Antigen-3 and Human Kallikrein-2 as Diagnostic Biomarkers for Prostate Cancer

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ABSTRACT

Background: Compared to nearby normal or benign tissues, Prostate cancer antigen-3 (PCA3) is significantly overexpressed in cancerous prostatic tissues. This biomarker has potential use in targeted treatment and clinical diagnostics.

Objective: To evaluate the role of PCA3 and human glandular kallikrein 2 (hk2) as diagnostic markers in prostatic cancer cases.

Subjects and methods: This study included one-hundred patients who were divided into 2 groups: fifty patients with benign prostatic hyperplasia and fifty patients with PC in addition to fifty healthy subjects as a control group. All were exposed to a thorough history, clinical examination, and detection of total PSA, free PSA, real-time PCR for PCA3, and hK2 mRNA.

Results: The cancer group had considerably greater PCA3 and hK2 levels compared to the BPH and control groups ($P<0.001$). The optimal PCA3 threshold for diagnosing PC was 6.1, with an area under curve of 0.954, sensitivity of 92%, specificity of 96%, positive predictive value of 95.8%, negative predictive value of 92.3%, and overall accuracy of 94% ($P<0.001$). The optimal cutoff for hK2 RQ to diagnosis PC was 3.2, with an area under curve of 0.874, sensitivity of 84%, specificity of 72%, positive predictive value of 75%, negative predictive value of 18.2%, and overall accuracy of 78% ($P<0.001$).

Conclusion: PCA3 is a potential biomarker and noninvasive test for PC diagnosis since it has higher diagnostic specificity and sensitivity than hK2 and PSA. It can be used either by itself or in conjunction with total PSA to diagnose PC.

Keywords: Prostate cancer gene 3 (PCA3), Prostate cancer (PC), Prostate-specific antigen (PSA).

INTRODUCTION

Prostate cancer (PC) is the second most frequent cancer in males, with a predicted 1.4 million diagnoses and 375,000 deaths globally in 2020 ⁽¹⁾. Most people will recover completely, but a sizable percentage of men will have the illness deteriorate or spread to other regions of their bodies ⁽²⁾. Once metastasis begins, there is no going back; approximately 30% of patients live five years following diagnosis. Aside from that, metastatic PC appears to have increased in prevalence over the previous decade across all age groups and racial/ethnic groupings ⁽³⁾.

Clinicians find it difficult to distinguish between PC and benign prostatic hyperplasia (BPH) since the clinical indications are so similar. PC has a poor overall prognosis because to the lack of timely and effective diagnostic methods. Physicians must discover PC early in order to reduce mortality, enhance survival rates, and optimize the possibility of successful medicinal interventions ⁽⁴⁾.

Serum PSA is still used in most PC tests. The limited specificity of PSA restricts its use as a screening test and prevents needless biopsies, even if a high PSA level is probably associated with PC. However, additional conditions such BPH, prostatitis, and PC may be linked to elevated PSA levels ⁽⁵⁾.

Around 1995, researchers collaborated to discover prostate cancer antigen 3 (PCA3). Originally

known as differential display clone 3 (DD3). PCA3 expression is substantially greater in PC tissue than in surrounding benign or normal tissue. In terms of clinical diagnosis and targeted treatment, PCA3 is an encouraging biomarker. Overexpression of the PCA3 genes has been discovered in various studies. This gene is a fragment of noncoding messenger ribonucleic acid (mRNA), found on chromosome 9q21-22. Using PCA3 determination, normal prostate cells can be distinguished from cancerous ones with accuracy close to one hundred percent at the cellular level. Gene levels in prostate cellular material-containing tissues or fluids have been utilized for diagnostic purposes due to the overexpression of PCA3 by cancer cells ⁽⁶⁾.

In addition, a new biomarker for PC is human kallikrein-2, a kind of serine protease that shares 79% of its amino acid sequence with PSA. It is predominantly made in the prostate, where it secretes pro-enzymes that are activated into active enzymes outside of the cell. Blood, semen, saliva, and other body fluids include human kallikrein-2; moreover, 80% to 95% of hK2 in the blood is free. Numerous studies have demonstrated the value of serum hK2 in PC identification and prognosis ⁽⁷⁾.

The current study objective was to evaluate the role of PCA3 and hK2 as a valuable, promising diagnostic marker in prostatic cancer patients.

SUBJECTS AND METHODS

This investigation was carried out on patients who were attending the outpatient clinic of Urology Department in Assuit University Hospitals in addition to other healthy group subjects during the period from January 2023 to June 2024.

Subjects enrolled included one-hundred patients divided into 2 groups: fifty patients with PC (group I), fifty patients with BPH (group II), in addition to fifty healthy subjects (group III) as a control group. The mean age of patients was 68 year and that of the control group was 67 years.

Cases with the following criteria were included: adult patients above 50 years old with elevated PSA or suspicious DRE and proved to be PC or BPH by prostatic biopsy. Exclusion criteria: Patients with symptoms of acute or chronic prostatitis, patients who had a history of other cancer, alcohol use, those who had any history of chronic physical illness, and patients who refused to enroll in the study.

Full clinical assessment, which includes complete history taking, clinical examination (by urology specialists) and anthropometric measurements, was performed for all patients. Estimation of tumor grade was done according to **Epstein** ⁽⁸⁾. Estimation of Gleason score was done according to **MacLennan and Bostwick** ⁽⁹⁾.

Laboratory tests were conducted including; complete blood count (CBC), kidney function tests, urine analysis, total PSA, free PSA and real time PCR for PCA3 and hK2 mRNA. CBC was performed on Sysmex XN-1000 automated hematology analyzer, kidney function tests were done by Roche/Hitachi Cobas c 311 system, total and free PSA were assayed

on ARCHITECT i1000SR by chemiluminescent microparticle immunoassay (CMIA), real time PCR for PCA3 and hK2 mRNA was performed through several steps including RNA extraction, real-time RT and cDNA synthesis and finally DNA amplification and detection. All kits used were provided by Thermo Scientific.

Ethical approval:

This study has been approved by the Sohag Faculty of Medicine's Ethics Committee. Each participant signed a consent form after all information was received. Throughout its execution, the study complied with the Helsinki Declaration.

Statistical analysis

Data analysis was done statistically using SPSS version 27.0. The expression for quantitative data was mean±standard deviation (SD), range, and median. Frequency and percentage were used to represent qualitative data, which were compared by χ^2 -test. ANOVA test was used to compare numeric quantitative variables. Correlation coefficient (R) test was used to correlate two numeric quantitative variables. A significant p-value was defined as one that is equal to or less than 0.05.

RESULTS

Statistically significant differences were found between the studied groups as regards renal function tests (urea and uric acid), urine analysis (pyuria and bacteriuria), TPSA, free PSA, PCA3 and hK2, which were significantly higher in the cancer group. The free/total PSA ratio was significantly lower in the cancer group (Table 1).

Table (1): Comparison between the studied groups regarding kidney function tests, pyuria, bacteriuria, PSA, PCA3 and hK2

Parameters	Group I (N=50)	Group II (N=50)	Group III (N=50)	P value
Urea (mg/dl) Mean $\pm$ SD Range	47.3 $\pm$ 8.5 35 – 62	43.2 $\pm$ 5.9 35 – 54	32.4 $\pm$ 6.8 23 – 52	< 0.001 (HS)
Uric acid (mg/dl) Mean $\pm$ SD Range	4.4 $\pm$ 0.62 3.5 – 6.0	4.3 $\pm$ 0.47 3.6 – 5.3	3.8 $\pm$ 0.47 2.8 – 5.0	0.001 (HS)
Pyuria 0 – 5/HPF More than 5/HPF	28 (56%) 22 (44%)	30 (60%) 20 (40%)	46 (92%) 4 (8%)	< 0.001 (HS)
Bacteriuria Nil ++	26 (52%) 24 (48%)	30 (60%) 20 (40%)	48 (96%) 2 (4%)	< 0.001 (HS)
TPSA Median Range	14.3 10.9 – 18.5	9.1 8.6 – 10.6	1.5 0.9 – 2.0	< 0.001 (HS)
Free PSA Median Range	2.8 2.4 – 3.2	2.2 2.0 – 2.5	0.3 0.2 – 0.5	< 0.001 (HS)
Free/total PSA Median Range	20.0 14.8 – 24.8	26.0 23.3 – 29.3	20.0 18.1 – 26.6	< 0.001 (HS)
PCA3 Median Range	8.6 7.5 – 10.3	4.2 3.2 – 5.5	0.3 0.05 – 0.6	< 0.001 (HS)
hK2 Median Range	4.3 3.5 – 7.1	2.3 2.1 – 3.7	0.3 0.05 – 0.8	< 0.001 (HS)

Regarding PCA3, there was significant positive correlation between PCA3 and TPSA ($r=0.516$) and between PCA3 and hK2 ($r=0.514$) and there was significant negative correlation between PCA3 and F/T PSA ($r=-0.46$) (Table 2 and Figures 1, 2, and 3).

Table (2): Correlation between PCA3 and the studied parameters among PC patients

Variable	(R)	P value
TPSA	0.516	0.008
F/TPSA	- 0.46	0.02
hK2	0.514	0.009

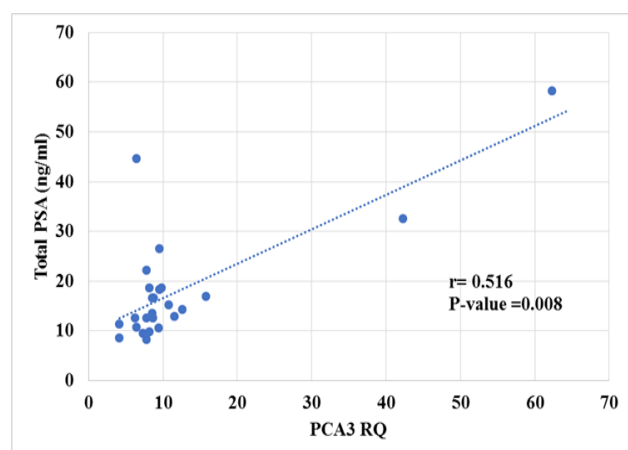


Figure (1): Correlation between PCA3 and TPSA among PC patients.

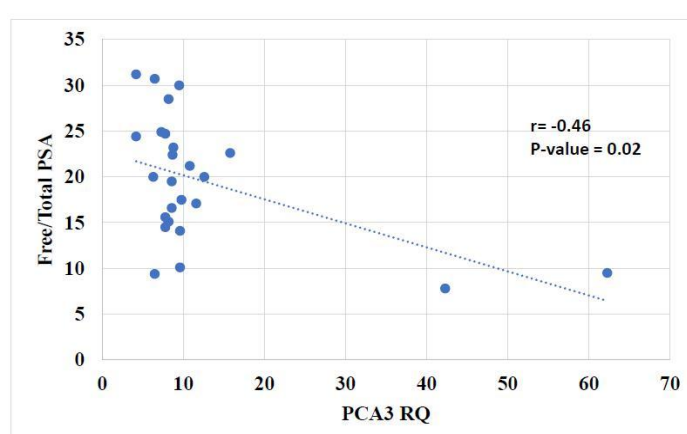


Figure (2): Correlation between PCA3 and F/TPSA among group I

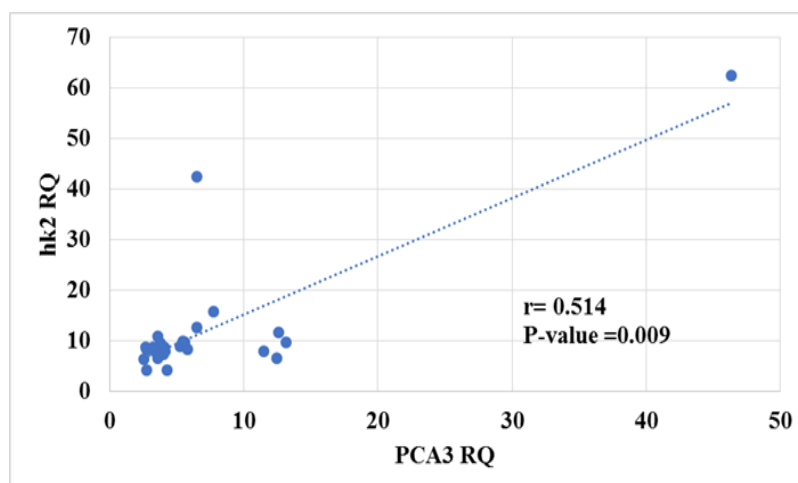


Figure (3): Correlation between PCA3 and hK2 among PC patients.

Regarding hK2, there was significant positive correlation between hK2 and TPSA ($r=0.516$) and significant negative correlation between hK2 and F/T PSA ($r=-0.64$) (Table 3 and Figures 4 and 5).

Table (3): Correlation between hK2 and the studied parameters among PC patients

Variable	(R)	P value
TPSA	0.516	0.008
F/TPSA	- 0.64	< 0.001

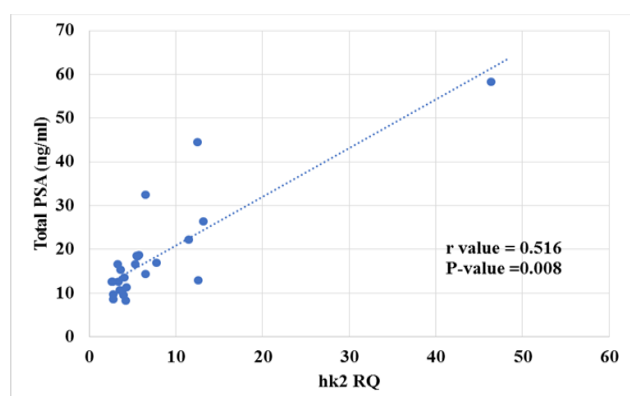


Figure (4): Correlation between hK2 and TPSA among PC patients.

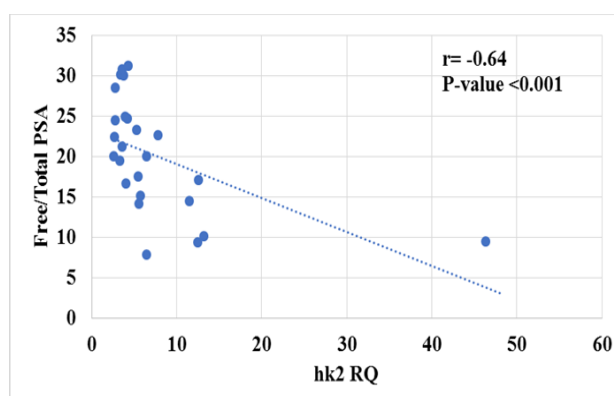


Figure (5): Correlation between hK2 and F/TPSA among PC patients.

The best cutoff for PCA3 relative quantitation (RQ) was 6.1. This cutoff had a diagnostic sensitivity of 92%, specificity 96%, and a significant AUC of 0.954. The best cutoff for hK2 (RQ) was 3.2. This cutoff had a diagnostic sensitivity of 84%, specificity 72%, and a significant AUC of 0.874. Total PSA at the cutoff 10.3 ng/ml had a diagnostic sensitivity of 84%, specificity 72%, and a significant AUC of 0.875. At cut off 24.8, free/total PSA had a diagnostic sensitivity of 76%, diagnostic specificity 56%, and a significant AUC of 0.780 (Table 4 and Figures 6-9).

Table (4): Performance of total PSA, F/T PSA, PCA3 and hK2 in diagnosis of PC

Parameter	TPSA	F/T PSA	PCA3 RQ	hK2 RQ
Cutoff	10.3	24.8	6.1	3.2
Sensitivity (%)	84	76	92	84
Specificity (%)	72	56	96	72
AUC (95% CI)	0.875 (0.77 – 0.98)	0.780 (0.65 – 0.91)	0.954 (0.894 – 1)	0.874 (0.781 – 0.966)
P-value	<0.001	<0.001	<0.001	<0.001
PPV (%)	75	63.3	95.8	75
NPV (%)	81.8	70	92.3	18.2
Accuracy (%)	78	66	94	78

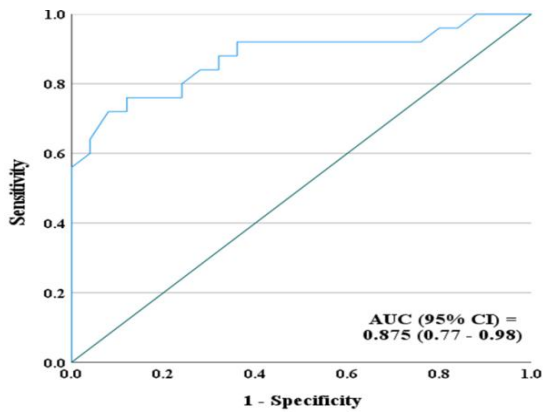


Figure (6): ROC curve analysis showing the diagnostic performance of total PSA.

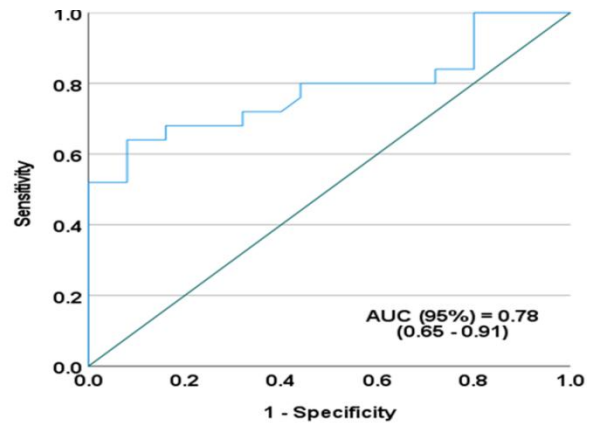


Figure (7): ROC curve analysis showing the diagnostic performance of F/T PSA.

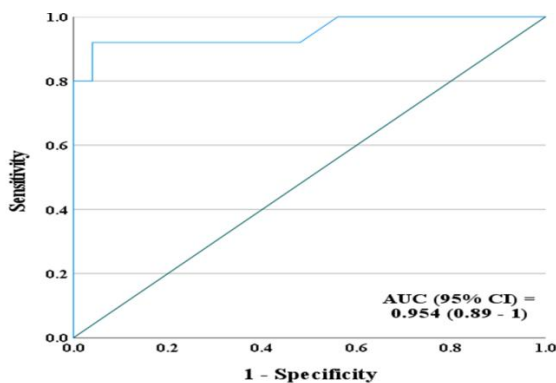


Figure (8): ROC curve analysis showing the diagnostic performance of PCA3.

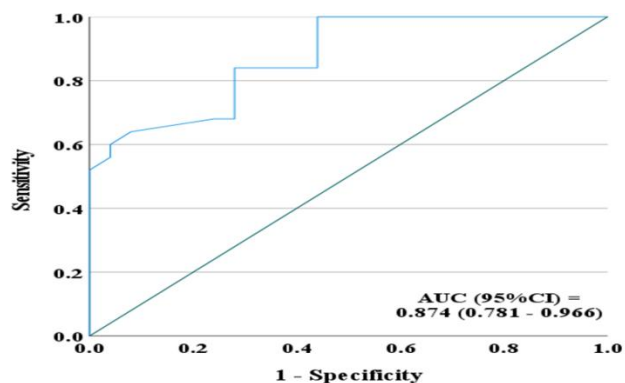


Figure (9): ROC curve analysis showing the diagnostic performance of hK2.

DISCUSSION

PC is the second most frequent cancer among males, behind lung cancer, and the third most common cancer globally. In 2020, there were about 0.4 million PC-related fatalities and 1.4 million new cases ⁽¹⁰⁾.

In the current study, there was significant increase in urea and uric acid levels between the patient groups and the control group ($P < 0.001$ and 0.001 respectively) that agree with results of **Oluboyo et al.** ⁽¹¹⁾ and **Pal et al.** ⁽¹²⁾.

The bulk of the tumor is strongly correlated with the blood level of urea, as has been shown in several types of cancer. Increased non-protein nitrogenous compounds have been linked to cancer cachexia, which causes skeletal muscle loss as a result of decreased synthesis and increased protein breakdown. These might be explained by the patients' lack of physical activity and the reduced availability of amino acids, which are instead used to create acute phase proteins ⁽¹³⁾. Antioxidant qualities are present in uric acid. Increased oxidative stress brought on by inflammatory bodily processes can harm cellular components, including deoxyribonucleic acid (DNA), which can contribute to the development of cancer. Due to its ability to scavenge reactive oxygen species and prevent lipid peroxidation, uric acid was thought to have a role in the basic defensive mechanism. Allopurinol, an inhibitor of xanthine oxidoreductase, is

one of few pharmacologic medications that are only used to treat hyperuricemia ⁽¹⁴⁾. **Lai et al.** ⁽¹⁵⁾ found a favorable connection between PC and allopurinol usage.

As regard urine analysis, there was significant pyuria and bacteriuria in the patient groups compared to the control group ($P: 0.01$ and 0.04 respectively) that agree with **Tolani et al.** ⁽¹⁶⁾ and **Akinpelu et al.** ⁽¹⁷⁾ who found that bacteriuria was shown to be 40.6% common in PC patients. These patient categories are more likely to develop bacteriuria due to urine stasis, poor bladder emptying, and urethral instrumentation such as catheterization and cystoscopy. Furthermore, age-related declines in zinc-associated antimicrobial components and a rise in prostatic fluid alkalinity might impact urinary tract bacterial colonization ⁽¹⁸⁾.

In this study, serum TPSA concentration was significantly higher in patients with cancer prostate when compared to BPH and control groups ($P < 0.001$). This was in accordance with **Ahmed et al.** ⁽⁶⁾ and **Yazdani et al.** ⁽¹⁹⁾ who found a significant difference between TPSA values in PC patients, BPH, and control groups. In a healthy prostate, the prostatic epithelium secretes PSA into the secretory ducts, where it contributes to the seminal fluid. Furthermore, in PC, the basal-cell layer is disrupted, allowing PSA to seep into the bloodstream and raise serum PSA levels ⁽²⁰⁾.

The findings of this investigation revealed that

patients with cancer prostate had lower mean for f/t PSA ratio than those with negative biopsy. Similar results were obtained by **Ahmed et al.** ⁽⁶⁾ and **Roddam et al.** ⁽²¹⁾ who found significant decrease in f/t PSA in PC patients than patients with BPH ($P < 0.001$). Study has found that as the f/t PSA ratio decreases, the probability of having cancer increases ⁽²²⁾.

As regards PCA3, the current study's findings showed highly significant increase in PC patients when compared to the other study groups ($P < 0.001$). These findings were consistent with **Ahmed et al.** ⁽⁶⁾ and **Lamouki et al.** ⁽²³⁾ who demonstrated that mean PCA3 was significantly higher in patients with PC. On the other hand, **Yazdani et al.** ⁽²⁴⁾ found non-significant difference in PCA3 between 14 patients with PC and 12 patients with BPH ($P = 0.199$). This may be due to small sample size. PCA3 is expressed 66-100 times more in PC cells than in normal prostate tissue. In comparison to benign tissue, it is also abundantly expressed in PC tissue. PCA3 expression is 140 times higher in cancer cells than in BPH, according to several studies ^(25,26). These data together suggest that PCA3 is overexpressed in PC and serves as a particular biomarker for this kind of cancer.

Additionally, the relationship between PCA3 and tumor aggressiveness as measured by Gleason score was examined. PCA3 showed a highly significant positive correlation with Gleason scores (P value: 0.001), where the highest levels were observed in patients with higher Gleason scores. These results were strengthened by **Ahmed et al.** ⁽⁶⁾, **AbdelSattar et al.** ⁽²⁷⁾, **Chunhua et al.** ⁽²⁸⁾ who reported the association between PCA3 levels and the severity of PC as defined by their Gleason score. Results of this study regarding increased PCA3 in advanced tumor grade and Gleason score suggest that PCA3 increase is associated with advanced disease.

In the present study, hK2 mRNA expression showed significant increase in group I when compared to groups II and III (P value < 0.001). This finding is in accordance with **Musavi et al.** ⁽²⁹⁾. Human glandular kallikrein 2 is an androgen-regulated protein that is almost exclusively produced in prostatic epithelial cells and shares 80% amino acid sequence similarity with PSA (hK3). Immunohistochemical investigations have revealed an incremental rise in hK2 expression from benign epithelium to prostatic intraepithelial neoplasia (PIN) to PC, despite the fact that PSA expression is lower in poorly differentiated PC than in well-differentiated malignancy. These features of hK2 imply that it may constitute a target to identify patients with PC from people with BPH and detect circulating, more biologically active PC cells ⁽²⁹⁾.

As regards Gleason scores, this study found significant increase in hK2 in group with high GS when compared to groups with lower GS ($P = 0.006$). The same observed by **Meola et al.** ⁽³⁰⁾ who found significant positive correlation between hK2 and

Gleason score.

In the present study, ROC curve analysis revealed that PCA3 had the highest AUC compared to TPSA, free PSA and hK2. AUC for PCA3 was 0.920 whereas it was 0.810, 0.786 and 0.687 for TPSA, free PSA and hK2 respectively. Similar findings were reported by **Ahmed et al.** ⁽⁶⁾, **Mao et al.** ⁽⁷⁾, **Merola et al.** ⁽²⁶⁾, **AbdelSattar et al.** ⁽²⁷⁾ and **Marks et al.** ⁽³¹⁾. However, **Adam et al.** ⁽³²⁾ found that AUC for TPSA beat PCA3 on ROC analysis. The difference in sensitivity, specificity and cutoff levels between the current study and the other studies is attributed to the difference in the size of the studied populations and the characteristics of this population. Indeed, the decision to enroll only individuals with a given risk for PC, such as increased PSA or an abnormal DRE, or based on the number of prior biopsies, would differ from the results obtained by screening the broader population. In addition to the difference in the assay and the quantitation method used (Maxima SYBR Green qPCR Master Mix, Thermo Scientific).

CONCLUSION

Since the current study identified PCA3 as a sensitive and specific biomarker for PC, it was evident that PCA3 is a potential marker of PC. Moreover, PCA3 can be applied as a prognostic marker as its levels correlated with the degree of PC severity using Gleason score. Therefore, our study emphasizes the use of PCA3 as a trustworthy marker for directing early biopsy decisions.

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No conflict of interest.

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