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A Comparative Study of Prostate Specific Antigen (PSA) and Prostate Cancer Antigen 3 (PCA3) Levels in Serum Of Prostate Cancer And Benign Prostatic Patients

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Abstract

Background: Prostate cancer ranks as the second most frequently diagnosed cancer and the fifth major cause of cancer-related deaths among males. Studies on prostate cancer biomarkers derived from prostate specific antigen have showed insufficient sensitivity and specificity for diagnosing and predicting in clinical settings. Furthermore, other genetic markers, including which more accuracy to detect of prostate cancer.**Patients and methods:** The research encompasses 90 participants, that include 30 patients of benign prostate hyperplasia, 30 of prostate cancer and 30 one of healthy individuals. The blood sample was obtained from each participant and utilized for the assessment of prostate-specific antigen and prostate cancer antigen 3 through the Enzyme-Linked Immunosorbent Assay (ELISA) method, following the manufacturer's guidelines supplied by Sun Long Biotech Company.**Results:** The present investigation demonstrated a statistically significant variance in prostate-specific antigen levels between groups. In prostate cancer patients was (5.36 ± 1.97), in benign prostatic hyperplasia was (3.62 ± 1.25) and in healthy individuals was (22.83 ± 0.965).prostate cancer antigen 3 levels in Prostate cancer, , benign prostatic hyperplasia nd healthy groups were (36.02 ± 10.55), (27.83 ± 6.05), and (18.55 ± 5.30), respectively and prostate cancer antigen 3 show high specificity (91%) in patients with Prostate cancer. **Conclusion:** The study reveals that prostate cancer antigen 3 is more effective and more specific in detecting prostate cancer than prostate-specific antigen.

***Key words*:** Prostate cancer (PCA), benign prostatic hyperplasia (BPH), prostate-specific antigen (PSA), prostate cancer antigen 3 (PCA3).

Introduction

Prostate cancer is currently the most often diagnosed cancer in men and the third leading cause of male mortality[1]. Prostate cancer ranks as the second most common cancer among males and the fourth most common cancer worldwide (Siegel et al., 2021; Sung et al., 2022). The annual incidence rates of advanced-stage prostate cancer increased by 4% to 6% from 2014 to 2018 (Schatten, 2018). In 2020, over 1.4 million individuals worldwide were diagnosed with prostate cancer (Sung et al., 2021)[2]. leading to 1,414,000 new cases and 375,304 deaths, accounting for 3.8% of all cancer-related fatalities in men. Prostate cancer is the fifth most commonly diagnosed malignancy and the seventh leading cause of cancer-related mortality in Asia[3].

Recognizing the risk factors of prostate cancer is crucial for implementing primary and secondary prevention strategies. The WHO has indicated that between 30%–50% of cancers may be preventable by mitigating risk factors and employing evidence-based preventive measures, including considerations of age, family history, race, and poor dietary practices and behaviors. Smoking is an independent behavioral risk factor for prostate cancer, with current smokers anticipated to exhibit an increased risk of prostate cancer mortality[4] .Prostate cancer is associated with the ageing process. In the United States, 70% of prostate cancer cases are diagnosed in men aged 65 years and older. Prostate cancer diagnosis is uncommon in men aged 50, but the incidence and mortality rates rise significantly[5].Family history is a recognized risk factor for the occurrence of PCA and may also elevate the chance of its fatal forms. Establishing the correlation between documented family history and PCA risk is complicated by variables including familial circumstances, recollection bias, and the extent of screening[6].

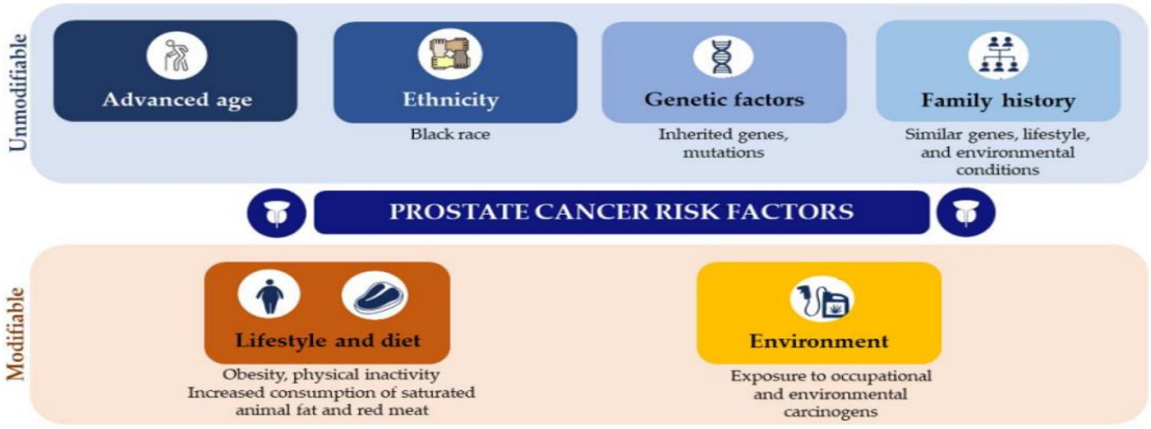


Figure -1 Modifiable and unmodifiable prostate Modifiable and unmodifiable prostate cancer risk factors. cancer risk factor[7].

The conclusive diagnosis of prostate cancer (PCA) relies on the findings from a prostate biopsy. Given the consequences linked to prostate biopsy, such as hospitalization, hemorrhage, infection, and discomfort, it is crucial to minimize the incidence of unneeded biopsies. For over three decades, the determination to perform a biopsy on a patient has depended about elevated prostate-specific antigen (PSA) values or anomalous findings from a digital rectal examination (DRE)[8]. Recent results indicate that the majority of routinely accessible prostate-specific antigen (PSA) derived biomarkers for prostate cancer (PCA) have not demonstrated adequate sensitivity and specificity in clinical applications for the diagnosis and prognosis of prostate cancer[9]

The primary limitation of PSA accuracy is its intrinsic variability due to numerous causes that can induce transitory increases not linked to cancer PSA fluctuations over a brief period are ascribed to assay variability, encompassing both analytical and biological variances. Analytical variation primarily arises from laboratory processing and assay performance. Biological variance arises from individual characteristics such as diurnal and circadian fluctuations, physical and sexual activity, urinary tract infections, and digital rectal examinations (DRE) [10].So because of these limitations of PSA and to reduce the un necessary biopsy, this study may be found other gen in serum sample which is possible more specificity for prostate cancer diagnosis like prostate cancer gen 3 (PCA3).

Prostate cancer gene 3 (PCA3) is an overexpressed long non-coding RNA (lncRNA) derived from an intronic region on the long arm of human chromosome 9q21–22. PCA3 influences prostate cancer (PCA) cell viability by modifying androgen receptor (AR) signaling and modulating the expression of various androgen-responsive and cancer-associated genes, including markers of epithelial–mesenchymal transition (EMT) and those pertinent to gene expression and cellular signaling[11]. Prostate cancer gene 3 (PCA3) is a noncoding mRNA found in prostate secretions from the first urine sample taken post-prostate massage. de Kok et al. showed that PCA3 is considerably overexpressed in prostate cancer tissue relative to normal prostate tissue still, The principal limitation of PSA is its insufficient specificity. In individuals with borderline PSA values of 3 to 10 ng/ml, the negative biopsy rate exceeded 60%, resulting in needless prostate biopsies and preventable consequences[12]

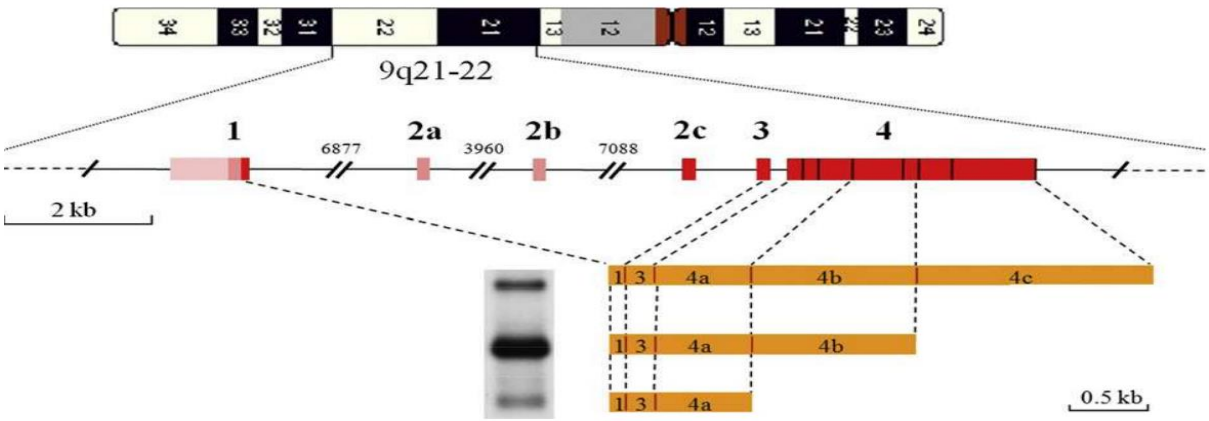


Fig. 2. The PCA3 gene is located on chromosome 9q21–22. The PCA3 transcription unit, as initially detailed by Busse makers et al .The gene comprises four exons (designated as boxes 1, 2c, 3, and 4) and features three polyadenylation sites situated within exon 4 (identified as boxes 4a, 4b, and 4c). Exon 2, often excluded as a result of alternative splicing, is absent in the three transcripts shown in the Northern blot. Clarke et al. identified two novel exons (2a and 2b), four new polyadenylation sites (indicated by vertical lines in exon 4), and four new transcription start sites (located in exon 1, represented by pink and light pink boxes). Gratitude is extended to Gerald Verhaeghe[13].

Patients and Methods

Study Design

This comparative study evaluated three groups: prostate cancer (PC), benign prostatic hyperplasia (BPH), and healthy individuals. The study aimed to assess PSA levels and PCA3 in the blood of patients from the Iraqi population.The study included 30 patients diagnosed with prostate cancer and 30 patients diagnosed with benign prostatic hyperplasia (BPH).

Control Group:

Thirty blood samples were collected from ostensibly healthy men with no history or clinical indications of prostatic illness. These samples were used as the control group.The participants, aged 36 to 82, were recruited from the Anbar Specialized Centre for Oncology and private urology clinics in Ramadi under the supervision of specialized physicians. The study was conducted from December 2023 to February 2024, with ethical approval granted by the Iraqi Ministry of Health and the University of Anbar Approval Committee (No. 666, dated 22/11/2023). Informed written consent was obtained from all participants prior to sample collection, in compliance with ethical research principles.

Exclusion and Inclusion Criteria

The study's inclusion criteria encompassed participants aged 18 years or older with pathological and cytological confirmation of prostate cancer (PC) or benign prostatic hyperplasia (BPH) who had not received prior therapy, while exclusion criteria ruled out individuals under 18 years of age, those who were HIV-positive, had a history of other malignant tumors, or presented with infectious diseases.

Material and methods:

Five ml of blood samples were collected from patients and the control group. Blood samples were placed in a gel tube and centrifuged to obtain serum for the quantification of PSA and PCA3 using an ELISA kit that use the Sandwich-ELISA test. ELISA procedure was accomplished aaccording to the instructions of the kit from Sun Long Biotech Company.

Statistical analysis: The data underwent statistical analysis with SPSS for Windows. version 26 (SPSS Inc., Chicago, Illinois, United States) The information was shown as a mean and standard deviation (SD). The parameters under study were checked to see if they adhered to a Gaussian distribution using the Shapiro-Wilk normality test. One way ANOVA test was done to show the differences between study groups (benign, tumor, and cancer) including a control group.. The area under the curve (AUC) of the ROC test was conducted to assess the specificity and sensitivity of the study parameters in relation to the study samples.

Results :

Mean PSA levels are highest in the Prostate Cancer group (5.36 ng/mL), followed by the Benign group (3.62 ng/mL), and lowest in the Healthy group (2.83 ng/mL).The p-value for the comparison between groups is (0.0001), indicating a statistically significant difference in PSA levels among the groups. While the Mean PCA3 levels are also highest in the Prostate Cancer group (36.02), followed by the Benign group (27.83), and lowest in the healthy group (18.55).The p-value for the comparison is (0.0001), suggesting significant differences in PCA3 levels across the groups. A p-value < 0.05 confirms significant differences in both PSA and PCA3 levels among the groups as shown in (table .1).

Table -1: showed Mean±SD and differences between two patients and control groups by paired t-test.(ANOVA).

Variables	Groups	Mean	Std. Deviation	Minimum	Maximum	p-value
PSA	Healthy	2.83	0.965	1.35	5.02	0.0001
	Prostate cancer	5.36	1.971	1.91	8.73	
	Benign	3.62	1.250	1.64	6.01	
PCA3	Healthy	18.55	5.302	11.73	33.42	0.0001
	Prostate cancer	36.02	10.558	13.49	50.85	
	Benign	27.83	6.054	17.75	41.59	
p-value less than 0.05 is significant differences						

This study findings regarding the level of PSA and PCA3 between two groups: Prostate Cancer and healthy individuals. The mean level of PS in Prostate Cancer group was 5.36±1.97 while in healthy group was 2.83±0.965 ng/mL with p-value (0.0001) indicating a statistically significant difference in PSA levels between the two groups. While PCA3 mean Level in Prostate Cancer group were 36.02±10.55 and in healthy group was 18.55±5.30 with p-value (0.0001) demonstrating a significant difference in PCA3 levels between the two groups as shown in (table .2).

Table-2: show the level of PSA and PCA3 in prostate cancer patients and control group.

Variables	Prostate cancer	Healthy	p-value
	Mean ± SD	Mean ± SD	
PSA	5.36± 1.97	2.83 ± 0.965	0.0001
PCA3	36.02 ± 10.55	18.55 ± 5.30	0.0001
p-value less than 0.05 is significant differences			

The results show that PSA and PCA3 levels in benign hyperplasia of prostate were higher than control group. PSA Levels in Benign group were 3.62±1.25 ng/mL ,while in healthy group were 2.83±0.965 ng/mL with p-value: 0.045, indicating a statistically significant difference in PSA levels between the two groups.

The level of PCA3 in Benign group were 27.83±6.05 and in healthy group were 18.55±5.30 with p-value: 0.0001, showing a highly significant difference in PCA3 levels between the groups as shown in (table .3)

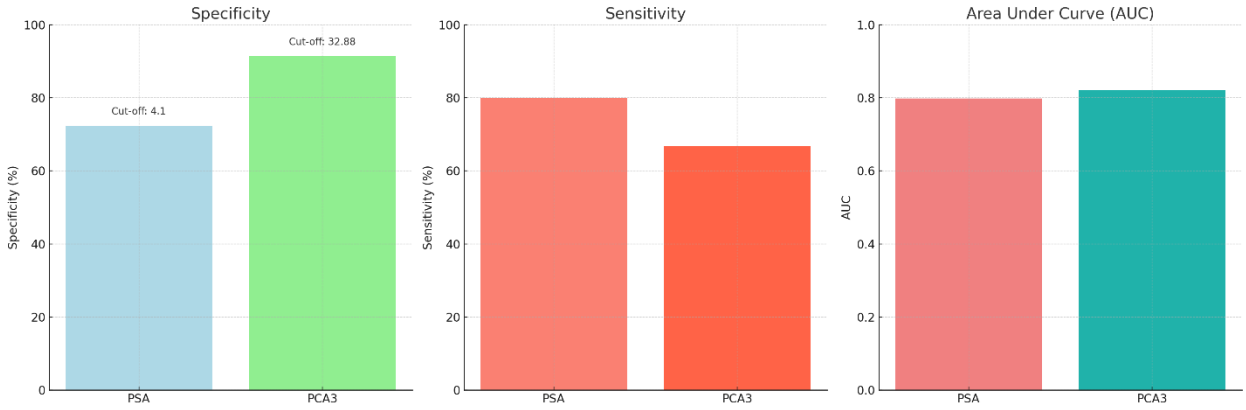
Table-3: show the level of PSA and PCA3 in benign hyperplasia patients and control group.

Variables	Benign	Healthy	p-value
	Mean ± SD	Mean ± SD	
PSA	3.62 ± 1.25	2.83 ± 0.965	0.045
PCA3	27.83 ± 6.05	18.55 ± 5.30	0.0001
p-value less than 0.05 is significant differences			

The results indicate the diagnostic performance of two biomarkers (PSA and PCA3) based on cut-off values, specificity, sensitivity, area under the curve (AUC), and statistical significance. At a cut-off value of 4.10, PSA has a sensitivity of 80% and a specificity of 72.4%, meaning it correctly identifies 80% of true positive cases (sensitivity) and 72.4% of true negatives (specificity). The AUC of 0.798 indicates good diagnostic performance, with a significant p-value (0.0001) while the PCA3 has a high specificity (91.4%) at the cut-off value of 32.88, indicating strong accuracy in ruling out false positives. However, its sensitivity is lower (66.7%), meaning it identifies about two-thirds of true positive cases. The AUC of 0.821 demonstrates good diagnostic performance, with a significant p-value (0.0001) as showed in (table.4)

Table-4: This table show the specificity and sensitivity of two biomarkers

Parameter	Cut-off value	Specificity	Sensitivity	Area-under curve	Sig.
PSA	4.10	72.4%	80%	0.798	0.0001*
PCA3	32.88	91.4%*	66.7%	0.821	0.0001*



(Figure.3) showed provided seems to contain information about the performance of two biomarkers—PSA (Prostate-Specific Antigen) and PCA3 (Prostate Cancer Antigen 3)—in detecting prostate cancer. PCA3 show high Specificity (91%) while PSA (72.4%).

Discussion

Prostate specific antigen (PSA), secreted by the prostatic epithelium, is organ-specific rather than cancer-specific, indicating that other diseases, including prostatitis and benign prostatic hyperplasia (BPH), as well as androgen levels, might affect PSA levels. A PSA level of 4 ng/mL is seen indicative of potential PCA, while levels ranging from 4 to 10 ng/mL are classified as ambiguous. Due to this specificity regarding PSA, there are dangers of overdiagnosis and overtreatment, leading to significant and unwarranted consequences. Evidence indicates that as many as 25% of patients with normal PSA levels may have undetected prostate cancer[14].

Therefore, in order to confirm a positive test result for prostate cancer, prostate infection, or other diseases, this study used multiple indicators in patients and controls. The present investigation demonstrated a statistically significant variance in PSA levels in prostate cancer patients (5.36 ± 1.97), followed by benign prostatic hyperplasia (3.62 ± 1.25) and the healthy group have (2.83 ± 0.965). The variation is high as indicated by the p value (0.0001). PCA3 levels show a statistically significant variance among the PC, PBH, and healthy groups (36.02 ± 10.55), (27.83 ± 6.05), and (18.55 ± 5.30), respectively, with a p value of 0.0001. This study found a notable disparity in the levels of PCA3 in the serum. ROC analysis was conducted to evaluate the clinical sensitivity and specificity of PCA3 and the sequential combination. Prostate cancer antigen 3(PCA3) is highly specific to prostate cancer due to its significant overexpression in prostate cancer cells. PSA is better suited for initial screening because of its higher sensitivity (80%), which reduces the risk of missing positive cases. Prostate cancer antigen3(PCA3) is more effective when accuracy in excluding false positives is a priority (specificity of 91.4%).Both tests are statistically significant with strong performance ($p = 0.0001$), but PCA3 has a slight edge in overall accuracy (AUC = 0.821).The PCA3 score and AUC for prostate cancer (PCA) in this study align with the findings of the Cui et al. meta-analysis [15].Prostate cancer antigen 3 (PCA3) serves as an alternative biomarker for prostate tumor specificity, potentially enhancing the specificity of prostate cancer diagnosis; nonetheless, it typically necessitates complex procedures and costly equipment for routine detection [16]. Prostate cancer antigen 3 (PCA3) is a long non-coding RNA that is extensively recognized as a prostate cancer biomarker, identified in tissue and, notably, in noninvasive samples[17].Our research found PCA3 transcripts, demonstrating that PCA3 specificity for cancer is virtually flawless due to the significant overexpression of the gene by cancer cells. In clinical testing for early prostate cancer, increased specificity is shown in serum containing prostate cells from affected males. PCA3 gene testing has significant potential in cases of increased PSA levels where initial biopsies reveal no malignancy. Peripheral blood from people with prostate cancer. Although not detected in all individuals with prostate cancer, no amplification was observed in benign prostatic hyperplasia samples, demonstrating the high specificity of PCA3.

Conclusion,

PSA and PCA3 serve complementary roles in the detection and differentiation of prostate cancer (PC) from benign prostatic hyperplasia (BPH). PSA is a widely accessible and cost-effective first-line screening tool but lacks specificity, often leading to false positives due to elevated levels in both PC and BPH. PCA3, with its higher specificity for PC, is a valuable secondary test that improves diagnostic accuracy, particularly in cases with elevated PSA levels and negative biopsies. The combined use of PSA and PCA3 enhances clinical decision-making, reducing unnecessary biopsies and ensuring more precise identification of prostate cancer.

Limitations

Future studies should aim to include larger, more diverse populations, standardize testing protocols, and evaluate PSA and PCA3 alongside other emerging biomarkers. Incorporating real-world data and cost-effectiveness analyses will also improve the relevance of research findings to clinical practice.

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