Differentially Expressed Blood ARLNC1 in Combination with PCA3/PSA has Reassuring Clinical Applications in the Early Diagnosis of Prostate Cancer in Iranians: A pilot study

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Purpose: Prostate cancer (PCA) is the second most common malignancy in Western countries. Long non-coding RNAs are new markers in disease diagnosis. Our aim of this study was to investigate liquid biopsy biomarkers with high specificity and sensitivity for early diagnosis of PCA patients in Iran.

Materials and methods: Blood specimens were collected from 29 PCA, 32 benign prostate hyperplasia (BPH), and 29 control (CTRL) individuals. Real-time PCR analyzed expression amounts of PSA, ARLNC1, UCA1, and PCA3. The ROC curve (receiver operating characteristic curve) analysis evaluated the diagnostic power of the examined molecules for PCA.

Results: There was a significant upregulation of PCA3 in PCA and BPH groups compared to the controls (p values for PCA3=< 0.001 and BPH vs. CTRL = 0.0015) while there was no significant difference between PCA and BPH individuals. A significant upregulation of ARLNC1 was seen in BPH group compared to the controls (*p* value = 0.0042). Also, PCA3 expression level showed a significant relationship with prostate volume.
There was no significant difference in UCA1 and PSA expression levels among the three groups (> 0.05). The PCA3/PSA ratio was significantly increased in PCA and BPH individuals vs. the CTRL group with high sensitivity and specificity. The gene expression of PCA3 and ARLNC1 in the BPH group showed a significant relationship with age.

Conclusion: Our findings showed that in the diagnosis of prostate cancer, measuring the expression of PCA3, PSA, and ARLNC1 genes is necessary to determine the health, benign, or cancerous status of patients' prostate. Also, selecting the PCA3/PSA ratio provides a new approach for diagnosing this cancer if confirmed in a larger clinical sample size and functional studies.

Keywords: radical retropubic prostatectomy; 3D modeling; positive surgical margin

INTRODUCTION

Prostate cancer is a highly heritable disease, the second most commonly occurring cancer in men[(1)], and the fifth death reason due to men's cancer[(2)]. Various factors such as smoking[(3)], diet[(4)], genetics and race[(5)], family history[(6)], obesity[(7)], environmental chemicals[(8)], prostatitis/inflammation of the prostate[(9)], sexually transmitted infections (STIs)[(10)], steroid hormone levels[(11,12)] and possibly vasectomy[(13)] are considered risk factors for the disease. Because of the heterogeneity of the disease, still a single gene or a genetic signature that can be attributed to the cause of prostate cancer has not been introduced[(14)]. The use of PSA as a marker for prostate cancer screening is complicated because its levels are significantly affected by factors such as race and age [(15,16)]. Unlike many other tumor markers, PSA levels do not correlate with prostate cancer progression[(17,18)] and metastasis[(19-26)]. Therefore, the identification of new more sensitive, and specific biomarkers is needed to detect prostate cancer in the early stages without the need for multiple biopsies[(27)]. Of course, even if NGS is performed, due to the variety of genetic changes in the prostate tissue, it is not possible to accurately and completely identify and classify the effective changes in PCA by using the genetic profile of patients[(28)].
Various cellular pathways are controlled by genes encoding receptors, co-receptors, enzymes, or RNA regulators, whose imbalance leads to pathway function in favor of tumorigenesis. Therefore, studying the expression level of these molecules can be useful in finding biomarkers for the diagnosis of prostate malignancy and its exact mechanism of proliferation, apoptosis, differentiation, invasion, or angiogenesis.

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Table 1. The sequences of the primers pairs for Real-Time PCR analysis.

Gene	Primer sequence (5'-3')	Size of Amplicon(bp)	Annealing Temperature
GAPDH-F	GGAAGGTGAAGGTCGGAGTCA	101	60°C
GAPDH-R 5	GTCATTGATGGCAACAATATCCACT		
PSA-F	ACCAGAGGAGTTCTTGACCCCAAA	161	60°C
PSA-R 1	CCCCAGAATCACCCGAGCAG		
UCA1-F	TCGGGTAACTCTTACGGT	109	58°C
UCA1-R 2	GGTCCATTGAGGCTGTAG		
PCA3-F	exon3-4: ACACAGGAAGCACAAAAGG	68	60°C
PCA3-R 3	GATGACCCAAGATGGCGGC		
ARLNC1-F	CCTTGTCCACTGGAACTCGT	100	58°C
ARLNC1-R 4	TATAACCTTGGGGGCCATGA		

Notes: All primers bind to either RNA or DNA except UCA1 primers that are specific to RNA. We decreased the extension time for PCA3 primers to prevent their activity on DNA. GAPDH and ARLNC1 primers could amplify DNA with a product size of ~ 3000bp which was not detected on gel electrophoresis.

The role of LncRNAs as novel biomarkers, in case of dysregulation, has been introduced in several human diseases including testicular cancer[29], breast cancer [30], and blood cancers[31]. DD3PCA3, a lncRNA/ a urinary/ and blood biomarker, is introduced for PCA presumptive diagnosis, risk stratification, and carcinogenesis [32,33,34]. A direct correlation of increased PCA3 concentration with higher degrees of tumor invasiveness has been shown. Combining PCA3 with new biomarkers increases the accuracy of disease diagnosis and prognosis. Compared to total PSA and total free PSA, PCA3 has a higher diagnostic value in identifying high-risk individuals. Also, In prostate cancer cells, PCA3 regulation depends on androgen receptor (AR) activation because gene expression changes have been reported only in AR-producing cell lines[35]. Unlike PSA, PCA3 is significantly increased in the neoplastic prostate and is not affected by prostatitis and prostatic hyperplasia [36]. PCA3 controls PCa cell survival by regulating AR markers and epithelial-mesenchymal transition (EMT) pathway markers[37] and decreases the survival of LNCaP cells following reduction[37].

ARLNC1 is located at 16q23.2 and has 4 exons[38]. It is a non-coding molecule under the control of the androgen receptor, which is strongly involved in the development and progression of prostate cancer[39]. ARLNC1 is not only induced by the AR protein but also stabilizes the AR transcript through RNA-RNA interaction. Deletion/inactivation of ARLNC1 inhibited AR expression, AR signaling, and prostate cancer growth in vitro and in vivo. Therefore, it seems that ARLNC1 helps to create positive feedback to AR signaling in the direction of prostate cancer progression, so it is considered a good target for the treatment of this disease[38].

UCA1 with 3 exons is located at chromosomal location 19p13.12. Its increased expression has been reported to be associated with advanced stages of cancer and poor prognosis[40]. Inhibiting or reducing the expression of

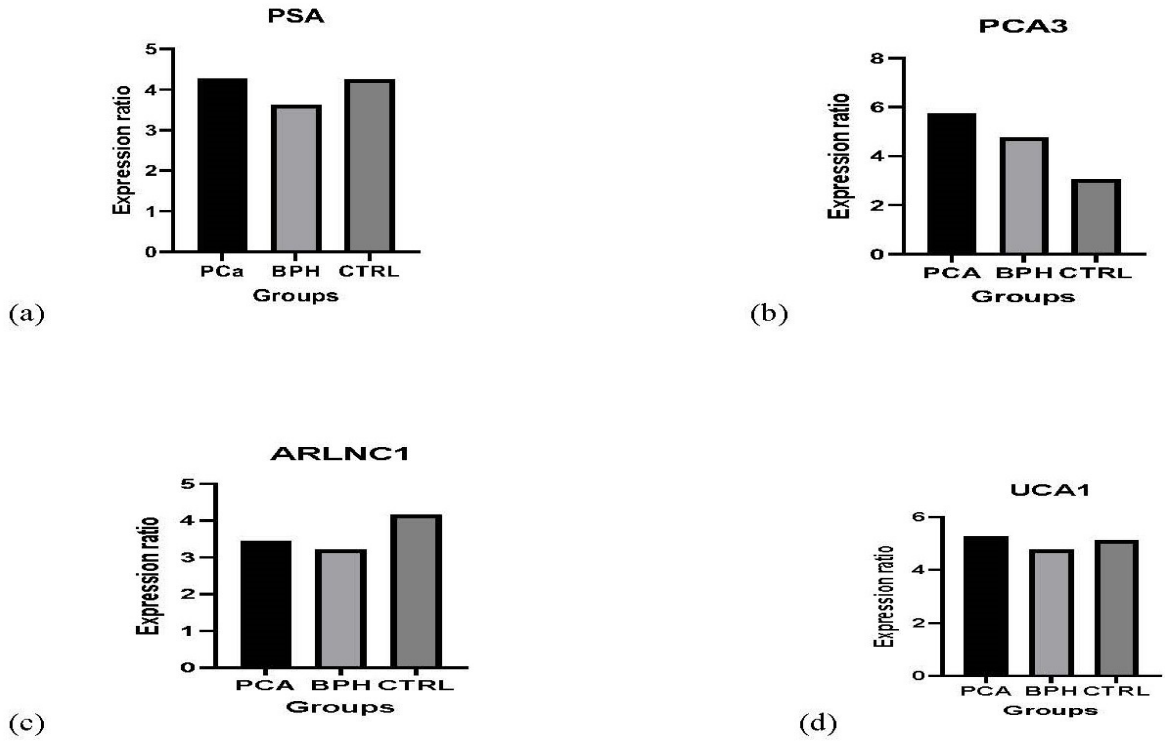

Figure 1. The relative mRNA expression level of under study genes in three different groups of PCA, BPH and CTRLs (n: 90).

Table2. Comparison of demographic and clinical characteristics between groups

Variables		Total	Group PCA (n=29)	BPH (n=32)	Control(n=29)	*P*-value
Age		66.7 ± 7.6	65.1 ± 5.4	67.8 ± 8.6	67.0 ± 8.4	0.309
Weight		78.5 ± 11.8	78.1 ± 12.4	78.9 ± 11.4	-----	0.807
Height		172.7 ± 6.1	172.8 ± 5.7	172.6 ± 6.6	-----	0.899
BMI		25.8 ± 3.3	26.1 ± 3.6	26.3 ± 2.6	25.0 ± 3.6	0.315
Gleason Score		7.5 ± 0.9	7.5 ± 0.9	-----	-----	
Size of tumor (cc)		47.4 ± 17.5	47.4 ± 17.5	-----	-----	
Prostate Volume and both seminal vesicles		60.1 ± 21.9	56.6 ± 15.8	66.1 ± 29.4	-----	0.277
PSA-ng/ml		7.0 ± 5.0	11.4 ± 6.0	6.0 ± 3.6	4.0 ± 0.0	
GAPDH-mean(ct)		20.6 ± 4.4	21.9 ± 6.2a	18.2 ± 1.7b	21.8 ± 3.3a	< 0.001
PSA mean(ct)		33.0 ± 2.2	32.9 ± 1.3a	32.3 ± 3.0a	33.8 ± 1.5b	0.012
PCA3 mean(ct)		24.0 ± 5.7	21.5 ± 3.8a	21.0 ± 3.5a	30.2 ± 4.3b	< 0.001
ARLNC mean(ct)		35.4 ± 4.6	36.1 ± 3.5	34.6 ± 2.6	35.5 ± 6.7	0.174
UCA1 mean(ct)		32.5 ± 5.1	33.3 ± 5.4	30.9 ± 5.2	33.4 ± 4.2	0.099
ARLNC efficiency		0.85 ± 0.54	-----	-----	0.85 ± 0.54	-----
Prostate cancer history	No	56 (62.22 %)	26 (89.66 %)	30 (93.75 %)		0.112
	Yes	3 (3.33 %)	3 (10.34 %)			
Other cancer history	No	53 (58.89 %)	23 (79.31 %)	30 (93.75 %)		0.011
	Yes	6 (6.67 %)	6 (20.69 %)			
Opium	No	63 (70.00 %)	21 (72.41 %)	20 (62.50 %)	22 (75.86 %)	0.736
	Yes	16 (17.78 %)	4 (13.79 %)	5 (15.63 %)	7 (24.14 %)	
Grade	3+3	1 (1.11 %)	1 (3.45 %)			-----
	3+4	11 (12.22 %)	11 (37.93 %)			
	4+3	6 (6.67 %)	6 (20.69 %)			
	4+4	2 (2.22 %)	2 (6.90 %)			
	4+5	3 (3.33 %)	3 (10.34 %)			
	5+3	1 (1.11 %)	1 (3.45 %)			
	5+4	2 (2.22 %)	2 (6.90 %)			
TNM STAGE	2	9 (10.00 %)	9 (31.03 %)			-----
	3	18 (20.00 %)	18 (62.07 %)			
Linear length of margin involves of carcinoma	bladder neck/perineural	5 (5.56 %)	5 (17.24 %)			-----
	no	7 (7.78 %)	7 (24.14 %)			
	perineural	6 (6.67 %)	6 (20.69 %)			
Lymphatic invasion	No	21 (23.33 %)	21 (72.41 %)			----
	Yes	4 (4.44 %)	4 (13.79 %)			
Vascular invasion	No	21 (23.33 %)	21 (72.41 %)			----
	Yes	4 (4.44 %)	4 (13.79 %)			
Perineural invasion	No	4 (4.44 %)	4 (13.79 %)			-----
	Yes	21 (23.33 %)	21 (72.41 %)			

Abbreviations: BMI, Body Mass Index; PSA, prostate specific antigen; TNM Stage, This system includes the extent of the tumor (T), extent of spread to the lymph nodes (N), and presence of metastasis (M).

Each subscript letter denotes a subset of Group categories whose mean values do not differ significantly from each other at the .05 level (for example the mean variables with subscript including a & ab and b & ab were not different. However, mean variables with subscript a and b was different)

UCA1 can be proposed as a new method in the possible treatment of prostate cancer patients[41]. Therefore, UCA1 can be a possible therapeutic target for PCA[42]. With the advancement of deep sequencing technology, several promising surrogate markers have been identified for PCa, including PCA3[43]. Therefore, the combined pattern of biomarkers as a genetic signature increases the specificity of the prostate cancer diagnosis algorithm[44].

In this research, we simultaneously examined the expression of PSA, PCA3, ARLNC1, and UCA1 in the blood of prostate cancer patients to determine their specificity and sensitivity individually or in combination in prostate cancer and benign prostatic hyperplasia compared to healthy individuals.

MATERIALS AND METHODS

Study Population

The definitive diagnosis of the disease was made based on the diagnosis of a urologist in Tehran hospitals, Iran. Demographic information was completed using a questionnaire. The blood samples were collected from patients with prostate cancer (PCA), men with BPH, and healthy individuals (controls).

Inclusion and exclusion criteria

PCA patients with PSA>10 were diagnosed by a urologist while their positive pathology results were sufficient in terms of the presence of neoplastic tissue, with or without a family history of the disease, without a history of radiotherapy, chemotherapy, or hormone therapy. Men with urinary symptoms, and benign prostatic hyperplasia (BPH), with a PSA value above 4 nanograms per milliliter (ng/ml) were biopsied based on magnetic resonance imaging (MRI) findings. These patients who showed increased epithelial cells in the microscopic examination of the biopsy and a negative pathological response for Prostate Cancer were included in the study as the BPH group. The patients with PSA less than 4 ng/ml, who were referred to the urology department of Medical Centers with abdominal pain and urinary symptoms without any prior history of surgery for the treatment of previous cancers, were selected as the control group. None of the subjects included in the study were subjected to a dual rectal exam (DRE). The samples are matched in terms of age and smoking status.

This study has been cleared by the SBMU Ethics Review Board for human studies and patients have signed an informed consent.

Procedures

RNA extraction and cDNA synthesis

In this study, 90 blood samples were tested and analyz-

Table 3. The relative mRNA expression level of PSA, PCA3, ARLNC1, and UCA1 in three different groups (PCA, BPH and CTRL. n: 90). Results were normalized with GAPDH gene. *P* Value < 0.05 is considered significant.

Dunn's multiple comparisons test		*P* Value	Adjusted *P* Value*
PSA	Case vs. CTRL	0.89	NO
	Case vs. BPH0.	16	NO
	CTRL vs. BPH	0.06	NO
PCA3	Case vs. CTRL	< 0.0001	YES
	Case vs. BPH0	.063	NO
	CTRL vs. BPH	0.0015	YES
ARLNC1	Case vs. CTRL	0.0699	NO
	Case vs. BPH	0.6420	NO
	CTRL vs. BPH	0.0042	YES
UCA1	Case vs. CTRL	0.2891	NO
	Case vs. BPH	0.3717	NO
	CTRL vs. BPH	0.9914	NO

Abbreviations: PSA, prostate specific antigen.

ed including 29 PCA, 32 BPH, and 29 men as controls. From each person, 5 ml of blood was collected under sterile conditions in EDTEA tubes and stored at -70 until use.

Total RNA was extracted from the specimens using an RNA extraction kit (Viragene, Iran) and the manufacturer's instructions. The quantity and quality of isolated RNA were checked by nanodrop spectrophotometry at 260 and 280 nm and electrophoresis on agarose gel. Single-stranded cDNAs were generated using the cDNA Synthesis Kit (Yekta Tajhiz Azma, Iran) as per the manufacturer's directions.

Evaluations

- Quantitative real-time RT-PCR

Quantitative Real-time PCR was performed on a RotorGene 6000 system (Corbett Research, Mortlake, Australia), using RealQ Plus 2x Master Mix Green (amplicon, Denmark) according to the manufacturer's protocol with the primer sets shown in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a normalizer and negative control were used in each run and all samples were examined in duplicate reactions. The thermal profile was 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and an annealing temperature for each primer set, for 30 s at 72 °C.

Gene expression was analyzed using the 2- ΔΔCT algorithm. Relative expression levels and fold changes were log2 transformed for data analysis. The results were analyzed as described below.

Statistical Analysis

To evaluate significant differences between gene expressions of the samples in two groups, a two-tailed Student's t-test using GraphPad Prism 8.0.2 (GraphPad Prism Software, Inc. San Diego CA, USA) was done. The assumptions underlying the independent t-test, including Normality and homogeneity of variance, were thoroughly evaluated. To determine the efficiency of

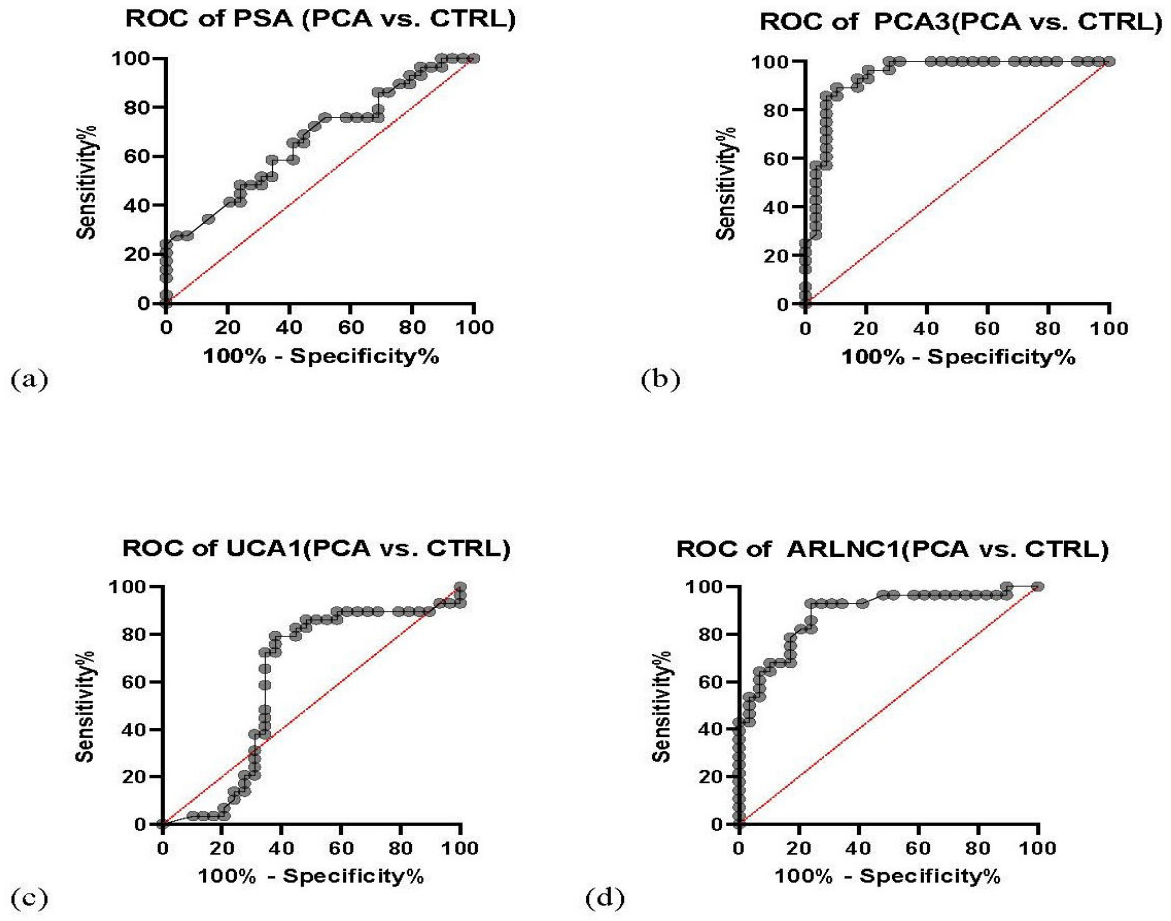

Figure 2. ROC curve analysis.

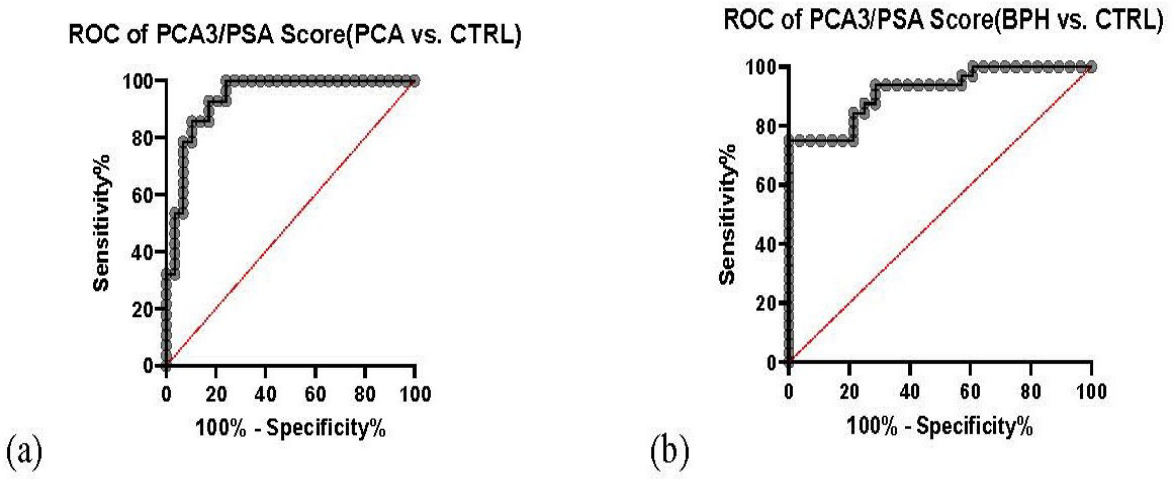

Figure 3. PCA3/PSA ratio ROC analysis between the groups.

the examined genes and PCA3/PSA ratio, receiver operating characteristic (ROC) curve analyses were performed by MedCalc-version19.1.2. In statistical analysis, a *P* value of <0.05 was considered significant. Also, the correlation between the expression level of the examined genes and other investigated factors was done with the help of Prism software and Pearson's r method.

RESULTS

• Patient Dataset

In total, 90 participants were included in the study with a mean ± standard deviation (SD) of 66.7 ± 7.6 years. Individuals were assigned to the three groups including PCA (29 cases), BPH (32 cases), and control (29 cases). The homogeneity of group and age ($p = 0.309$) and BMI (0.315) was observed in this research. Accordingly, significant relations were seen between groups and variables including GAPDH-mean ($p < 0.001$), PSA-mean ($p = 0.012$), and other cancer history ($p = 0.011$) (**Table 2**).

• Expression Variation of the Examined Genes

The expression of PSA, PCA3, ARLNC1, and UCA1 were evaluated by Real-Time PCR in three sets of samples. The mRNA expression level of PCA3 was significantly higher in PCAs and BPHs in comparison with the control group (*p* value = < 0.001). Besides, an increased expression of ARLNC1 was seen in BPH group compared to the controls (*p* value = 0.0042). However, the expression level of the other examined genes was not significant in the studied groups (**Table 2, 3**) (**Figure 1**).

• ROC Curve Analysis of Examined Genes

The receiver operating characteristic curve (ROC) was plotted to find the value of evaluating the expression of the under-study genes as biomarkers for prostate cancer diagnosis or prognosis. ROC analysis in PCA vs. CTRL showed area under the ROC curve of 0.66 for PSA (Sensitivity 48%, Specificity75%, 95% CI 0.5297 to 0.8068, and *P* value = 0.02), 0.94 for PCA3 (Sensitivity 89%, Specificity 89%, 95% CI 0.8813 to 1.000, and *P* value < 0.0001), 0.88 for ARLNC1 (Sensitivity 78%, Specificity 82%, 95% CI 0.7978 to 0.9743, and *P* value < 0.0001), 0.604 for UCA1 (Sensitivity 72%, Specificity 65%, 95% CI 0.4460 to 0.7633, and *P* value = 0.17).

Also, in PCA vs. BPH ROC analysis revealed an AUC of 0.63 for PSA (Sensitivity 65%, Specificity 65%, 95% CI 0.4882 to 0.7758, and *P* value = 0.07), 0.56 for PCA3 (Sensitivity 59%, Specificity 51%, 95% CI 0.4212 to 0.7113, and *P* value = 0.37), 0.66 for ARLNC1 (Sensitivity 62%, Specificity 65%, 95% CI 0.5310 to 0.8084, and *P* value = 0.02), 0.65 for UCA1 (Sensitivity 65%, Specificity 62%, 95% CI 0.5179 to 0.7935, and *P* value = 0.03).

ROC analysis in BPH vs. CTRL showed AUC of 0.76 for PSA (Sensitivity 93%, Specificity 62%, 95% CI 0.6354 to 0.8872, and *P* value = 0.0005) , 0.95 for PCA3 (Sensitivity 89%, Specificity 90% , 95% CI 0.9004 to 1.000, and *P* value < 0.0001), 0.80 for ARLNC1 (Sensitivity 64%, Specificity 84%, 95% CI 0.6859 to 0.9168, and P value < 0.0001), 0.78 for UCA1 (Sensitivity 72%, Specificity 84%, 95% CI 0.6572 to 0.9139, and *P* value = 0.0001) (Table S1,S2 and S3), (**Figures2, S1 and S2**)

• Correlation analysis between tested genes and patients' demographic characteristics

In the results related to investigating the correlation between the expression level of each gene and factors such as age, weight, height, prostate volume, PSA(ng), stage, size of tumor and grade, no significant relationship was found for PCA3, ARLNC1 genes but the correlation of UCA1 gene with the stage of the prostate cancer, r: -4.772, 95% CI -7225 to -0.112, P(two-tailed): 0.0129 and PSA ng/ml, r: -0.3902, 95% CI -0.6754 to -0.003303, P(two-tailed): 0.0488 and PSA with stage r: -0.4573, 95% CI -0.7133 to -0.09352, P(two-tailed): 0.0165 were significant (Table S4) (FigureS3).

• PCA3/PSA, ARLNC1/PSA and UCA1/PSA ratio difference analyses between groups and the related

ROC curve analysis

PCA3/PSA ratio difference analyses between groups were done by Dunn's multiple comparison tests and as a result, the ratio difference showed to be significant for PCA vs. CTRL and BPH vs. CTRL (**Table S5**), (**Fig. S4**). In the continuation, the ROC curve analysis was performed to achieve the diagnostic value of the PCA3/PSA ratio in PCA vs. CTRL containing the results AUC

0.93 (sensitivity 85%, specificity 89%, 95% CI 0.8766 to 1.000, and *p*-value < 0.0001) and in BPH vs. CTRL, AUC 0.91 (sensitivity 75%, specificity 100%, 95% CI 0.8497 to 0.9851, and *p*-value < 0.0001) (**Table S6**), (**Figure 3**). ARLNC1/PSA and UCA1/PSA ratio difference analyses between groups indicated no different significance (**Tables S7 and S8**) (**Figures S5 and S6**).

DISCUSSION

Prostate cancer is one of the most common diseases in men. Because the prostate tissue is a heterogeneous tissue, its early diagnosis sometimes requires multiple biopsies. In this research, we tried to find a clue of a genetic signature to prevent unnecessary biopsies by examining the expression of several genes that were previously confirmed to be specific to the prostate, at the same time, in three groups of healthy people, with benign prostate hyperplasia, and those with cancer.

For this purpose, the gene expression levels of four PSA, PCA3, ARLNC1, and UCA1 genes were measured and their gene expression changes were investigated about prostate cancer. Regarding PCA3, in line with the results of other researchers such as Kewen Zheng, good results were obtained in using PCA3 gene expression as an acceptable and non-invasive diagnostic biomarker to distinguish prostate cancer patients from healthy individuals.[45] Considering the results related to the comparison of PCA3 gene expression between two PCA and BPH groups in this pilot study, the need to investigate the number of patients and therefore more data in future studies seems necessary. Considering the importance of the PCA3/PSA ratio mentioned in many articles[46,47], in the present study, the results of the comparison and statistical analysis of this ratio showed significant changes between PCA vs. CTRL and also between BPH vs. CTRL, which may have a high diagnostic value due to high sensitivity and specificity.

PSA is an important tumor marker for prostate cancer, which is induced by the Androgen receptor (AR) and is regulated at the transcriptional level[48], other known factors, including P53, are negative regulators of this gene[49]. For the past few years, the level of PSA protein in blood serum has been measured to diagnose a patient with prostate cancer, but considering that a low PSA cannot guarantee that a person does not have prostate cancer, we evaluated the level of the gene expression and its changes between the three studied groups. Our results demonstrated that although the level of expression increased in the PCA group compared to BPH, the difference was not significant, as was previously reported by V. Uma Bai and her colleagues[50]. However, as mentioned before, according to the practical results related to PCA3/PSA examination, measuring the expression level of PCA3 and PSA genes is helpful and necessary for a more accurate and timely diagnosis of prostate cancer[51,52].

As a long non-coding RNA, ARLNC1 is important for AR signaling and prostate cancer progression. AR induces ARLNC1 expression while ARLNC1 stabilizes AR transcript through RNA-RNA interaction. Various studies show that increasing the expression of ARLNC1 causes large tumors, and blocking this gene can inhibit AR expression, AR signaling, and prostate cancer growth[53]. Therefore, it is expected that the expression level of ARLNC1 is elevated in the PCA patients compared to BPHs and controls. In this study, we found a significant difference between the sample groups considering ARLNC1 expression level. Therefore, as Kamla Kant Shukla pointed out, confirming the role of the expression levels of this gene in the etiology of prostate cancer in the Iranian population requires further study [54].

As a long non-coding RNA, UCA1 plays a role in many types of cancer such as cervical, stomach, bladder, and osteocarcinoma, increasing cell proliferation and invasion by affecting microRNAs and other regulatory factors[55,56]. On the other hand, in the article published by Xian Zhao et al. in 2020, contrary to previous studies that emphasize the effect of the UCA1 promoter on tumorigenesis and its progression, it has been found that this lncRNA stabilizes the E-Cadherin protein with two mechanisms by preventing from the interaction between this protein with the E3 Ligase MDM2 and as a result suppressing the degradation of E-Cadherin on the one hand, as well as the function of UCA1 as a miR-296-3p sponge that targets the E-Cadherin gene at the post-transcriptional level, maintaining the level of E-Cadherin increases in level, and as a result, cancer prostate cells become less tumorigenic and non-metastatic at the initial level, so it can be said that a high level of UCA1 is not always threatening[57]. In the present study, the changes in the measured gene expressions were not significant in the three groups. However, according to the above explanations, and the significant correlation obtained between the level of UCA1 gene expression and the stage of the disease, as well as the level of PSA protein, further studies on the double effect of UCA1 on prostate cancer will be very valuable.

CONCLUSIONS

The significant relationship between the expression levels of ARLNC1, PCA3, PCA3/PSA and the pathogenesis of BPH and PCa in this research needs further studies and confirmation of a larger sample size.

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CONFLICT OF INTEREST

None declared by the authors.

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APPENDIX

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REFERENCES

1. Rawla P. Epidemiology of Prostate Cancer. World J Oncol. 2019 ;10:63-89.
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality

Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 ;71:209-49.
3. Jochems SHJ, Fritz J, Häggström C, Järvholm B, Stattin P, Stocks T. Smoking and Risk of Prostate Cancer and Prostate Cancer Death: A Pooled Study. Eur Urol. 2023 ;83:422-31.
4. Matsushita M, Fujita K, Nonomura N. Influence of Diet and Nutrition on Prostate Cancer. Int J Mol Sci. 2020 20;21(4).
5. Conti DV, Darst BF, Moss LC, et al. Trans-ancestry genome-wide association meta-analysis of prostate cancer identifies new susceptibility loci and informs genetic risk prediction. Nat Genet. 2021 ;53:65-75.
6. Vietri MT, D'Elia G, Caliendo G, et al. Hereditary Prostate Cancer: Genes Related, Target Therapy and Prevention. Int J Mol Sci. 2021 4;22(7).
7. De Pergola G, Silvestris F. Obesity as a major risk factor for cancer. J Obes. 2013;2013:291546.
8. Tse LA, Lee PMY, Ho WM, et al. Bisphenol A and other environmental risk factors for prostate cancer in Hong Kong. Environ Int. 2017 ;107:1-7.
9. Doat S, Marous M, Rebillard X, et al. Prostatitis, other genitourinary infections and prostate cancer risk: Influence of non-steroidal anti-inflammatory drugs? Results from the EPICAP study. Int J Cancer. 2018 ;143:1644-51.
10. Tantengco OAG, Aquino IMC, de Castro Silva M, Rojo RD, Abad CLR. Association of mycoplasma with prostate cancer: A systematic review and meta-analysis. Cancer Epidemiol. 2021 Dec;75:102021.
11. Friedenreich CM, Ryder-Burbidge C, McNeil J. Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. Mol Oncol. 2021 ;15:790-800.
12. Tafuri A, Sebben M, Pirozzi M, et al. Association between Basal Total Testosterone Levels and Prostate Cancer D'Amico Risk Classes. Urol Int. 2020;104:716-23.
13. Xu Y, Li L, Yang W, et al. Association between vasectomy and risk of prostate cancer: a meta-analysis. Prostate Cancer Prostatic Dis. 2021 ;24:962-75.
14. Konishi N, Shimada K, Ishida E, Nakamura M. Molecular pathology of prostate cancer. Pathology international. 2005;55:531-9.
15. Hinata N, Fujisawa M. Racial Differences in Prostate Cancer Characteristics and Cancer-Specific Mortality: An Overview. World J Mens Health. 2022 ;40:217-27.
16. Saw S, Aw T-C. Age-related reference intervals for free and total prostate-specific antigen in a Singaporean population. Pathology. 2000;32:245-9.
17. Hoefner DM. Serum tumor markers: part II--practical considerations and limitations of testing. MLO Med Lab Obs. 2006;38:10-1, 4.
18. Keetch DW, Catalona WJ, Smith DS. Serial prostatic biopsies in men with persistently elevated serum prostate specific antigen values. J Urol. 1994;151:1571-4.
19. Ward JF, Moul JW. Rising prostate-specific antigen after primary prostate cancer therapy. Nat Clin Pract Urol. 2005;2(4):174-82.
20. Vis A, Kranse R, Roobol M, Van Der Kwast TH, Schröder F. Serendipity in detecting disease in low prostate-specific antigen ranges. BJU international. 2002;89(4):384-9.
21. Schröder F, Wildhagen M, Cancer' RSGotERSoSfP. Screening for prostate cancer: evidence and perspectives. BJU international. 2001;88(8):811-7.
22. Kranse R, Beemsterboer P, Rietbergen J, Habbema D, Hugosson J, Schröder FH. Predictors for biopsy outcome in the European Randomized Study of Screening for Prostate Cancer (Rotterdam region). The Prostate. 1999;39(4):316-22.
23. Schröder FH, Van Der Cruijsen-Koeter I, De Koning HJ, Vis AN, Hoedemaeker RF, Kranse R. Prostate cancer detection at low prostate specific antigen. J Urol. 2000;163(3):806-12.
24. Schröder F, Wildhagen M. Low levels of PSA predict long-term risk of prostate cancer: results from the Baltimore longitudinal study of aging. Urology. 2002;59(3):462.
25. Frankel S, Smith GD, Donovan J, Neal D. Screening for prostate cancer. The Lancet. 2003;361(9363):1122-8.
26. Ornstein DK, Kang J. How to improve prostate biopsy detection of prostate cancer. Current Urology Reports. 2001;2(3):218-23.
27. Madu CO, Lu Y. Novel diagnostic biomarkers for prostate cancer. J Cancer. 2010 Oct 6;1:150-77.
28. Alarcón-Zendejas AP, Scavuzzo A, Jiménez-Ríos MA, et al. The promising role of new molecular biomarkers in prostate cancer: from coding and non-coding genes to artificial intelligence approaches. P Prostate Cancer Prostatic Dis. 2022 Sep;25(3):431-43.
29. Liu H, Xu Y, Yao B, Sui T, Lai L, Li Z. A novel N6-methyladenosine (m6A)-dependent fate decision for the lncRNA THOR. Cell death & disease. 2020;11(8):613.
30. Hassani B, Taheri M, Asgari Y, et al. Expression analysis of long non-coding RNAs related with FOXM1, GATA3, FOXA1 and ESR1 in breast tissues. Front Oncol. 2021;11:671418.
31. Wang F-Y, Gu Z-Y, Gao C-J. Emerging role of long non-coding RNAs in normal and malignant hematopoiesis. Chinese Medical Journal. 2020;133(04):462-73.
32. De Kok JB, Verhaegh GW, Roelofs RW, et al. DD3 PCA3, a very sensitive and specific marker to detect prostate tumors. Cancer research. 2002;62(9):2695-8.
33. Tinzl M, Marberger M, Horvath S, Chypre C. DD3PCA3 RNA analysis in urine–a new perspective for detecting prostate cancer. Eur Urol. 2004;46(2):182-7.
34. Ferreira LB, Palumbo A, de Mello KD, et al. PCA3 noncoding RNA is involved in the control of prostate-cancer cell survival and modulates androgen receptor signaling. BMC

cancer. 2012;12:1-15.
35. van Bokhoven A, Varella-Garcia M, Korch C, et al. Molecular characterization of human prostate carcinoma cell lines. The Prostate. 2003;57(3):205-25.
36. De Luca S, Passera R, Bollito E, et al. Comparison of prostate cancer gene 3 score, prostate health index and percentage free prostate-specific antigen for differentiating histological inflammation from prostate cancer and other non-neoplastic alterations of the prostate at initial biopsy. Anticancer Res. 2014;34(12):7159-65.
37. Lemos AEG, Ferreira LB, Batoreu NM, de Freitas PP, Bonamino MH, Gimba ERP. PCA3 long noncoding RNA modulates the expression of key cancer-related genes in LNCaP prostate cancer cells. Tumour Biol. 2016;37:11339-48.
38. Zhang Y, Pitchiaya S, Cieślik M, et al. Analysis of the androgen receptor-regulated lncRNA landscape identifies a role for ARLNC1 in prostate cancer progression. Nat Genet. 2018 Jun;50(6):814-24.
39. Yang Y, Liu KY, Liu Q, Cao Q. Androgen Receptor-Related Non-coding RNAs in Prostate Cancer. Front Cell Dev Biol. 2021;9:660853.
40. Yao F, Wang Q, Wu Q. The prognostic value and mechanisms of lncRNA UCA1 in human cancer. Cancer Manag Res. 2019;11:7685-96.
41. Yu Y, Gao F, He Q, Li G, Ding G. lncRNA UCA1 Functions as a ceRNA to Promote Prostate Cancer Progression via Sponging miR143. Mol Ther Nucleic Acids. 2020 Mar 6;19:751-8.
42. Hu M, Yang J. Down-regulation of lncRNA UCA1 enhances radiosensitivity in prostate cancer by suppressing EIF4G1 expression via sponging miR-331-3p. Cancer Cell Int. 2020;20:449.
43. Yang Z, Yu L, Wang Z. PCA3 and TMPRSS2-ERG gene fusions as diagnostic biomarkers for prostate cancer. Chin J Cancer Res. 2016 Feb;28(1):65-71.
44. Matuszczak M, Schalken JA, Salagierski M. Prostate Cancer Liquid Biopsy Biomarkers' Clinical Utility in Diagnosis and Prognosis. Cancers (Basel). 2021 Jul 5;13(13).
45. Zheng K, Dou Y, He L, et al. Improved sensitivity and specificity for prostate cancer diagnosis based on the urine PCA3/PSA ratio acquired by sequence-specific RNA capture. Oncol Rep. 2015;34(5):2439-44.
46. Wang R, Wu Y, Yu J, Yang G, Yi H, Xu B. Plasma messenger RNAs identified through bioinformatics analysis are novel, non-invasive prostate cancer biomarkers. Onco Targets Ther. 2020;13:541.
47. Moranova L, Stanik M, Hrstka R, Campuzano S, Bartosik M. Electrochemical LAMP-based assay for detection of RNA biomarkers in prostate cancer. Talanta. 2022;238:123064.
48. Saxena P, Trerotola M, Wang T, et al. PSA regulates androgen receptor expression in prostate cancer cells. The Prostate. 2012;72(7):769-76.
49. Ecke TH, Schlechte HH, Schiemenz K, et al. TP53 gene mutations in prostate cancer progression. Anticancer Res. 2010;30(5):1579-86.
50. Bai VU, Kaseb A, Tejwani S, et al. Identification of prostate cancer mRNA markers by averaged differential expression and their detection in biopsies, blood, and urine. Proc Natl Acad Sci U S A. 2007;104(7):2343-8.
51. Moránová L, Staník M, Hrstka R, Bartošíkm. Electrochemical Assay for Detection of Prostate Cancer Biomarkera in Urine. Book of abstracts; 2021; 2021. p. 100.
52. Mowayeiz KJ, Al-Wasiti EA, Hussein AG, Hasan NA, Al-Nasiri US. The Utility of PSA, PCA3 in Detection of Men with Prostate Tumors. Indian J Public Health. 2019;10(8).
53. Takayama K-i, Fujimura T, Suzuki Y, Inoue S. Identification of long non-coding RNAs in advanced prostate cancer associated with androgen receptor splicing factors. Commun Biol. 2020;3(1):393.
54. Shukla KK, Misra S, Sankanagoudar S, et al. Recent scenario of long non-coding RNAs as a diagnostic and prognostic biomarkers of prostate cancer. Urol Oncol: Seminars and Original Investigations; 2020: Elsevier; 2020. p. 918-28.
55. Yao F, Wang Q, Wu Q. The prognostic value and mechanisms of lncRNA UCA1 in human cancer. Cancer Manag Res. 2019;11:7685.
56. Ghafouri-Fard S, Taheri M. UCA1 long non-coding RNA: An update on its roles in malignant behavior of cancers. Biomed Pharmacother. 2019;120:109459.
57. Zhao X, Wang Y, He J, et al. LncRNA UCA1 maintains the low-tumorigenic and nonmetastatic status by stabilizing E-cadherin in primary prostate cancer cells. Mol Carcinog. 2020;59(10):1174-87.