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Evaluation of the urine mRNA-PCA3 expression level in prostate patients; comparison between benign prostatic hyperplasia and cancer

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Abstract

Introduction: Prostate cancer is among the most common malignancies in men which can be well-managed if early diagnosed. Nevertheless, to date, no accurate index has been detected in order to differentiate prostate cancer from the other benign conditions practically except biopsies that is an invasive procedure.
Objectives: The current study is aimed to assess the values of urinary prostate cancer antigen 3 (PCA3) in prostate cancer diagnosis.
Patients and Methods: This case-control study was conducted on 28 patients with elevated levels of prostate-specific antigen (PSA) who underwent transrectal prostate biopsies in 2019. The urinary level of PCA 3 was measured using real-time polymerase chain reaction (RT-PCR). Ultrasonography was performed to assess the volume of prostate, as well. PSA density (PSAD) was defined as PSA divided by prostate volume. The patients were divided into two groups of benign prostatic hyperplasia (BPH) or prostate cancer based on prostate biopsy pathological report. The values of PCA 3 were evaluated.
Results: PCA 3 had accuracy of 0.708 at cut-off point of 27.75 with measured AUC of 0.720 (95% CI: 0.510-0.931), sensitivity and specificity of 72% and 69%, respectively. PSA had accuracy, sensitivity, and specificity of 0.708, 70% and 71.4% at cut-off of 9, whereas PSAD had 0.667, 88% and 50% at cut-off of 4.8, respectively.
Conclusion: Based on the findings of this study, urinary PCA 3 can be considered as a valuable biomarker for the prediction of malignancy in prostate biopsies with the sensitivity and specificity of 72.7% and 69.2% at the cut-off level of 27.75, respectively.

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Introduction

Nowadays, prostate cancer is considered as the second commonly diagnosed type of cancer and the third most frequent cause of death from cancer in men (1). According to recent reports, the annual worldwide incidence rate of this cancer was estimated to account for 1.6 million persons per year (1,2). Therefore, finding an early predictor can play a substantial role in the management and prognosis of patients with this type of cancer (3).

Serum prostate-specific antigen (PSA) level has been widely used as the gold standard for the screening of prostate cancer (4). However, despite its high specificity for clinical applications, PSA is not a cancer-specific biomarker, as it can be affected by confounding factors like prostatitis or benign prostatic hyperplasia (BPH) (5). Therefore, numerous investigations are in progress on this factor including; PSA-related indices such as PSA isoforms, free/total PSA ratio and volume-referenced PSA in order to promote the specificity of the biomarkers in the detection of prostate cancer (6-8). Although the elevation of PSA can be associated with the risk of developing prostate cancer, as an invasive screening tool, unnecessary biopsies are likely required, which limits its applicability for routine clinical applications (9,10).

Key point

The early detection of prostate cancer can lead to proper management of this malignancy as one of the most common types of tumors worldwide. Although this goal can be achieved by screening, there is no specific and sensitive biomarker in this term. The current study aimed to assess prostate cancer antigen 3 (PCA3) for this regard and found the sensitivity and specificity of 72.7% and 69.2% at the cut-off level of 27.75, respectively.

Bussemakers et al introduced prostate cancer antigen 3 (PCA 3) as an untranslated prostate-specific messenger RNA (mRNA), which is over-expressed in prostate biopsies (11). Further investigations not only confirmed this finding in terms of this novel biomarker, but also have shown up to 66-folds over-expression of PCA3 in prostate cancer, compared to the normal or other benign conditions (12,13). In this regard, numerous studies have been conducted, or in progress, to evaluate the applicability of PCA 3 rather than PSA for detection of prostate cancer. Nevertheless, providing a significantly diverse study population, possibly multi-centric, may produce more accurate results on the cut-off values of this biomarker, which leads to higher predictive capability (10,14-17). Therefore, a pilot study in the community of Iran is required to determine a cut-off value for the application of PCA 3 among Iranian men with prostate cancer.

Objectives

The current study aimed to evaluate the urine mRNA-PCA3 expression level in prostate patients in Iran, in order to provide a cut-off value of this biomarker for the diagnosis of prostate cancer.

Patients and Methods

Study design

The current case-control study was conducted on 28 patients with elevated levels of PSA who underwent transrectal prostate biopsies in the affiliated urology clinics at AJA University of Medical Sciences from April 2019 to December 2019.

Over 40 years old men with increased levels of PSA and/or abnormal digital rectal examination (DRE) were included. Previous history of prostate cancer, urinary tract infections, history of any invasive treatment for BPH and administrations of any agent affecting the serum levels of PSA were considered as the exclusion criteria.

The patients who met the inclusion criteria were enrolled in the study through convenience sampling. One-by-one block sampling was performed to gather the data, in this term, parallel to a patient with the absolute diagnosis of prostate cancer based on prostate biopsies, a case with the diagnosis of BPH following ruling malignancy out was entered into the study as a control participant.

Diagnosis of prostate cancer

Extended prostate biopsy (12 cores) was conducted for all of the studied population based on the standard protocols for prostate biopsies (10). Besides, the blood sample was taken from the participants and serum PSA was measured. In addition, the volume of prostate was measured for all the participants using ultrasonography. The latter measurement of this study was PSA density (PSAD) calculated by the division of prostate volume by PSA levels.

The biopsies underwent pathological study and the percentage of positive cores was reported. By division of positive cores by the total number of taken cores, the percentage of the positive cores was calculated. Thereafter, the patients were divided into two groups (≤33% positive core and >33% positive cores) (18). Indolent cancer was determined as the Epstein criteria; PSAD <0.15, Gleason score ≤6 and less than six positive cores on a twelve-core biopsy (18).

Quantitative PCA 3 measurement

In order to gather urine specimens for PCA 3 evaluation, patients underwent a DRE and the first voided sample after that was examined using PROGENSA PCA3 assay. The quantitative PCA 3 was measured as follows;

Total RNA from urine sediments was extracted using TRIzol reagent (Invitrogen; No. 15596-026, USA). 50 ng of total mRNA was treated with with DNase I (TaKaRa: D2215, TaKaRa, Japan) prior to cDNA synthesis and then amplified with a TransPlex Complete Whole Transcriptome Amplification Kit (WTA2 Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Quantitative reverse transcription PCR (qRT-PCR) was performed using SYBR® Premix Ex Taq™ (TaKaRa: DRR081A TaKaRa, Japan) with an Applied BioSystems StepOne Plus according to the manufacturer's recommended cycling conditions. The gene-specific sequence information for the qRT-PCR primers is as follows; PSA-forward primer GTCTGCGGCGGTGTTCTG, PSA-reverse primer TGCCGACCCAGCAAGATC; PCA3 forward primer TGGTGGGAAGGACCTGATGATACAG, and PCA3 reverse primer TCTCCCAGGGATCTCTGTGCTTCC. Briefly, 2 µl of the cDNA solution was amplified using 10 µl SYBR® Premix Ex Taq™ (Perfect Real Time) (2×) (TaKaRa: DRR081A TaKaRa, Japan), 2 µl primers, 0.4 µl ROX Reference Dye (50×), and nuclease-free H2 O in a final volume of 20 µl. The data were analyzed with StepOne Software version v2.1 (Applied BioSystems, USA). A melt-curve analysis was performed at the end of the amplification. Samples with PSA cycle threshold (Ct) values of >28.15 were excluded to ensure sufficient prostate cell collection. All of the assessments were performed twice. The signals' amplifications were not obtained by the addition of nuclease-free water was added instead of cDNA. StepOne software version v2.1 (Applied BioSystems, USA) was utilized to analyze the data (15).

Statistical analysis

Descriptive statistics were reported as mean± standard deviation for continuous variables with normal distribution or median (inter-quartile range) for those with non-normal distribution and frequency (percentage) of patients for categorical variables. The independent sample t-test was used to measure the difference of the mean score between cancer and BPH groups. In addition, the PCA3 performance was assessed using the area under

the curve (AUC) of the receiver-operating characteristics and measures of diagnostic accuracy including sensitivity, specificity and accuracy value. The optimal cut-off point of the model was determined using Youden index, calculated as (sensitivity+ specificity–1). The level of statistical significance was considered at $P=0.05$. All statistical analysis was conducted using IBM SPSS Statistics version 21.

Results

In the current study, data of 28 patients, including 14 ones with prostate cancer and 14 ones with BPH were recruited among which, two persons with BPH were excluded due to incomplete medical records.

The mean age of the cancerous cases was 67.07 (4.85) years and the patients with BPH was 62.81 (8.06) years which revealed non-significant difference between the groups ($P=0.087$). In addition, the studied population were not statistically different in terms of PSA level ($P=0.172$), PSAD ($P=0.055$), and urine PCA3 levels ($P=0.199$); but the prostate volume was remarkably higher among BPH cases as compared to the patients suffering from prostate cancer ($P=0.014$). The detailed information is presented in Table 1.

Based on Table 2, among the studied prostate related indices, PCA 3 had the highest level of accuracy at cut-off point of 27.75 with measured AUC of 0.720 (95%CI: 0.510-0.931), sensitivity and specificity of 72% and 69%, respectively. At the same time, PSA and PSA density represented accuracy of 0.708 and 0.667 at the cut-off points equal to 9 and 4.8, respectively. Detailed information is presented in Table 2 and Figure 1.

Discussion

To the best of our knowledge, there is no study on the evaluation of the urine mRNA-PCA3 expression level in prostate patients in Iran, for the detection of cancer among those with abnormal levels of PSA who underwent transrectal prostate biopsies. According to our findings, patients diagnosed prostate cancer have shown remarkably higher serum PSA (19), and fee-to-total PSA levels (20), prostate volume (21) and also PSA density (21). Although the remarkable higher levels of mentioned factors among the cancerous people in comparison to BPH are in line with the previous studies (20, 21), to definite cancer diagnosis, trans-rectal biopsies were also required (22).

PCA3 gene which is located in zones 21–22 of no. 9 human chromosome with a total length of 25 kb and composed of four exons and three introns has been introduced by Baltimore and colleagues (23). Investigations of this gene revealed elevated levels of this gene expression among patients with prostate cancer (24). By further studies, it was revealed that PCA3 gene expression is elevated not only in blood but also all the

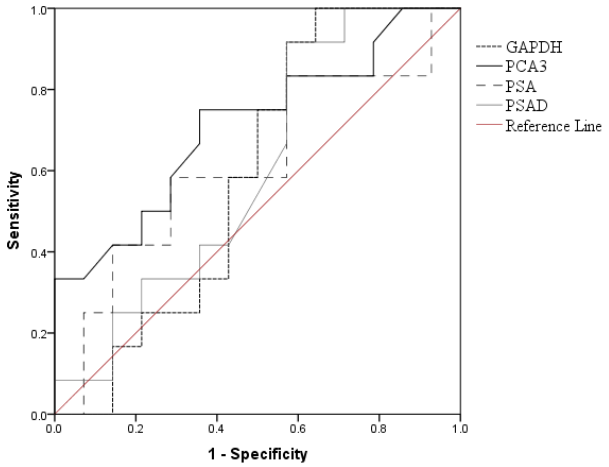

Figure 1. The ROC of diverse prostate-related indices for early diagnosis of malignancy.

Table 1. Demographic and clinical characteristics of the two studied groups

	Prostate cancer (n=14)	BPH (n=12)	*P* value
Age (years), mean ± standard deviation	67.07±4.85	62.81±8.06	0.087
PSA (ng/mL)	21.64±27.79	10.85±4.28	0.172
Prostate volume (cc)	36.82±12.74	58.39±26.67	0.014
PSAD	3.71±5.41	5.91±3.33	0.055
GAPDH (Ct mean)	26.04±3.26	25.70±4.46	0.823
PCA3 (Ct mean)	29.45±3.09	27.95±2.64	0.199
ΔCt	3.41±3.58	2.26±4.13	0.457

Table 2. The diagnostic values of diverse prostatic indices for the differentiation of BPH versus prostate cancer

	Cut point	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
GAPDH	28.5	0.591 (0.363-0.819)	1.00 (0.715-1.000)	0.357 (0.127-0.648)	0.640 (0.425-0.820)
PCA3	27.75	0.720 (0.510-0.931)	0.727 (0.390-0.939)	0.692 (0.385-0.909)	0.708 (0.489-0.873)
PSA	9	0.650 (0.409-0.891)	0.700 (0.347-0.933)	0.714 (0.419-0.916)	0.708 (0.489-0.873)
PSAD	4.8	0.648 (0.409-0.887)	0.888 (0.517-0.997)	0.500 (0.211-0.789)	0.667 (0.430-0.854)

body fluids which led to the assessment of urinary levels, a finding that was confirmed by the study of Long-Ya et al, that represented significantly increased levels of PCA3 mRNA in both blood and prostatic secretions of patients with prostate cancer as compared to BPH (25).

We evaluated diverse prostatic indices values in differentiation of prostate cancer from BPH, which revealed the superiority of PCA3 mRNA than the other more popular ones, such as PSA or PSAD. This outcome was in line with the previous studies evaluating the values of different indices in the early detection of prostate cancer (20, 21).

Based on our study, urinary assessment of PCA3 mRNA at the cut-off of 27.75 had the sensitivity and specificity of 72.7% and 69.2%, respectively. These findings were in line with the study of Li et al on Chinese people in 2018. Their study assessed the levels of PCA3 mRNA among patients with cancer, BPH and nephrolithiasis and found remarkable higher levels of gene expression among those with cancer than the other two pathological conditions. Further assessment led to determination of considerable sensitivity and specificity of 87.5% and 79.2% at the cut-off of 33.86(26).

Most of the other studies in the literature have defined an index, PCA 3 score which is calculated by the division of PCA3 mRNA by PSA mRNA. Ochiai et al assessed the values of PCA3 assay for the detection of prostate cancer and revealed promising outcomes at the cut-off value 35 as a valuable cut-off with specificity and sensitivity of 71.6% and 66.5% for the discrimination of cancerous masses from BPH, respectively (10). In another study with a similar design, Groskopf et al utilized PCA 3 assay and represented the sensitivity and specificity of 69% and 79% at the cut-off level of 50 among North American men (27). Deras et al have conducted another study in North America, which assessed the use of PCA 3 assay in urine samples for the diagnosis of prostate cancer. They showed that the cut-off level of 35 led to the ultimate 54% sensitivity and 74% specificity for the detection of prostate cancer. However, they stated that the addition of other indices, including DRE, serum PSA, and prostate volume to PCA 3 could potentially increase the AUC from 0.69 to 0.75. Therefore, they insisted on the necessity of a thorough clinical and para-clinical examination instead of using PCA 3 biomarker as a standalone determinant of prostate cancer (28). Gils et al investigated the value of derived PCA3 from urine samples versus prostate fluid following a prostate manipulation through DRE in the diagnosis of prostate cancer. They also collected the pathological findings obtained from prostate biopsy for the evaluations. They represented a cut-off level of 66 with the specificity and sensitivity of 82% and 65% from the prostate fluid, respectively; whereas, the urine specimens were accompanied by the ultimate values of 80% and 61% at the threshold level of 43 (29). Adam et al conducted their study among the African males and represented the highest values by the threshold of 60 with specificity and sensitivity of 68.9% and 66.7%, respectively (30).

Conclusion

Based on the findings of this study, urinary PCA3 can be considered as a valuable biomarker for the prediction of malignancy in prostate biopsies with the sensitivity and specificity of 72.7% and 69.2% at the cut-off level of 27.75, respectively. Due to the limited numbers of investigations regarding the use of urinary PCA3 mRNA for prostate cancer diagnosis, further studies are strongly recommended.

Limitations of the study

Full validation of the derived results needs inclusion of other comprehensive potential assays such as, the duration of the disease, the race and ethnicity of the patients and drug history to provide higher predictive capability. A significantly larger study population may produce more detailed results on the specificity and sensitivity of the urine levels of PCA3 for clinical applications.

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Authors' contribution

AY, NF, GS and HN were the principal investigators of the study. AY and HN were included in preparing the concept and design. AY and HN revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The institutional ethical committee at AJA University of Medical Sciences approved all study protocols (IR.AJAUMS.REC.1399.040). Accordingly, written informed consent was taken from all participants before any intervention. Additionally, Ethical issues (including plagiarism, data fabrication, double publication) were completely observed by the authors.

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