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Original Article

Diagnostic value of total serum/free prostate specific antigen and prostate cancer antigen-3 levels in prostate cancer

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Abstract: Background: The purpose of this study was to compare serum total and free prostate specific antigen (PSA) levels and serum prostate cancer antigen-3 (PCA3) levels in patients with prostate cancer in 2018 and 2019. Methods: This research was a prospective case-control study. The case group included all patients with suspected prostate cancer, and the control group included individuals without prostate disease who were referred to Ali Asghar and Nour Hospital in Isfahan, Iran, from October 2018 to October 2020. The serum total PSA, free PSA, and PCA3 levels in both groups were measured using the ELISA method with standard kits and compared between the groups. Results: The two groups were matched in terms of age and body mass index (BMI). The results showed that the mean free PSA level in the control group was significantly higher than that in the case group ($P<0.05$). Conversely, the mean total PSA level in the case group was significantly higher than that in the control group ($P<0.05$). However, no significant difference was observed in the mean PCA3 levels between the case and control groups. In addition, the total PSA variable with a cutoff of ≤3.14 exhibited 93% sensitivity and 82% specificity, demonstrating the highest diagnostic accuracy in distinguishing between prostate cancer and healthy individuals. Similarly, the PCA3 value with a cutoff of ≤3.5 had a sensitivity and specificity of 70% and 72%, respectively. Conclusion: Overall, the study results indicated that total PSA and PCA3 levels have higher diagnostic accuracy in distinguishing patients with suspected prostate cancer from healthy individuals.

Keywords: Prostate, screening, cancer, PCA3, PSA

Introduction

Prostate cancer is a prevalent malignancy in men and ranks as the second leading cause of cancer-related death among men in the United States. Its prevalence is higher in elderly individuals, with a global rate of eight cases per 100,000 people [1]. About three-quarters of cases occur in men over the age of 65, with an average age of 72 [2]. The incidence of prostate cancer is also increasing among Iranian populations [3]. Screening plays a crucial role in reducing the burden of this cancer [2, 4, 5]. Several factors, including ethnicity, diet, genetics, environmental factors, and inflammation, have been linked to the development of prostate cancer [6]. Genetic and epigenetic alterations can lead to the inactivation of tumor suppressor genes and activation of oncogenes [7]. While older age is a known risk factor, the precise mechanisms underlying the selective initiation of tumoral cells in the prostate compared to other genitourinary organs, as well as the expression of malignancy within the peripheral tissue of the gland, remain subjects of debate [8].

The higher incidence rate of prostate cancer in developed countries can be attributed primarily to the availability of advanced screening methods, such as prostate-specific antigen (PSA) and prostate cancer antigen-3 (PCA3) tests [9]. Evaluating both total and free PSA levels, as well as their ratio, is important for accurate assessment, particularly considering the impact of factors like race, age, and dietary habits

on PSA levels [10]. Serum free and total PSA levels are commonly used biomarkers for detecting and monitoring prostate cancer. PSA is produced by both normal and cancerous prostate cells, and elevated levels can indicate the presence of prostate cancer. However, PSA levels can also be influenced by non-cancerous conditions, leading to false-positive results. Serum free PSA, which is not bound to other proteins in the blood, is used in conjunction with total PSA to differentiate between prostate cancer and benign conditions. A lower percentage of free PSA relative to total PSA is associated with a higher risk of prostate cancer. In contrast, the PCA3 test is a newer biomarker specifically developed for prostate cancer diagnosis. The correlation between serum free and total PSA levels with serum PCA3 levels in prostate cancer patients can vary. Implementing age-specific PSA reference ranges can be a helpful approach to improve screening accuracy, particularly by considering an upper limit of four nanograms per milliliter [10]. It is important to note that PSA levels can be elevated in conditions other than prostate cancer, such as nodular prostate hyperplasia, while they can remain within the normal range in some cases of non-metastatic prostate cancer. Although the free/total PSA ratio of less than fourteen percent is generally applicable, it is particularly useful in cases where PSA levels are under four nanograms per milliliter [10].

PCA3 is a gene present in all prostate cells, and its down-regulation leads to reduced protein synthesis. The presence of PCA3 in urine can serve as an initial indicator of potential abnormalities [11]. Generally, men without pathological prostate findings do not have detectable levels of PCA3 [12]. This marker can be particularly useful in cases where there are elevated PSA levels but negative biopsy results, as it may indicate the presence of prostatitis [13]. Importantly, PCA3 levels remain unaffected by conditions that typically cause elevated PSA levels, such as infections and inflammations. Therefore, PCA3 testing can be utilized as an alternative to repeat biopsies, achieving a sensitivity level of ninety percent [14]. Given the significance of these considerations, this study aimed to compare serum free and total PSA levels with serum PCA3 levels in patients with prostate cancer and healthy control subjects.

Methods

Study design

This research was a prospective case-control study.

Population study

In this study, the case group consisted of all patients with suspected prostate cancer who were referred to Ali Asghar and Nour Hospital in Isfahan, Iran, from October 2018 to October 2020. The control group included individuals without prostate disease who were also referred to Ali Asghar and Nour Hospital during the same period.

Inclusion and exclusion criteria

The inclusion criteria for both groups, in addition to prostate status, included confirmation of benign prostate hyperplasia (BPH) and prostate cancer diagnosis by a pathologist through biopsy results. Participants were also required to provide consent to participate in the study and be over the age of 50. The study excluded individuals with symptoms of acute or chronic prostatitis, which were diagnosed based on clinical examination by a urologist and relevant tests. Individuals with a history of any cancer, alcohol use, or chronic physical illness were also excluded from the study. It is important to note that patients without a definitive cancer diagnosis were excluded from the study before data analysis took place.

Sampling method and sample size

The sampling method employed in this study was simple random sampling, whereby individuals meeting the eligibility criteria were selected until the desired sample size was reached.

Additionally, taking into account a 95% confidence level, α=0.05, 80% test power, and the provided values of S1=40, S2=42, M1=60, M2=34 (presumably representing standard deviations and means), the sample size for the study was determined based on similar studies. The calculated sample size for this study was 80 subjects, with 40 subjects allocated to each group. Considering a potential sample loss of 20%, the total sample size was set at

Table 1. Determination and compare the mean of free and total PSA and The PCA3 in control and case groups

Variables	Case group	Control group	*P*-value*
Free PSA levels	0.37±0.03	1.20±0.32	0.02
Total PSA levels	35.06±5.1	1.86±0.34	0.001
The PCA3 levels	7.70±1.1	8.47±1.8	0.7

*Using Independent T test.

100 individuals, with 50 individuals in each group.

Measurement method

The serum levels of PSA were measured in the laboratory using the CanAg PSA EIA test. For the assessment of PCA3 levels, 20-30 ml of urine samples were collected from the patients after a digital rectal exam. The collected urine samples were then stored at temperatures between 2-8 degrees Celsius. The measurement of PCA3 levels in the urine samples was conducted using the ELISA method, specifically utilizing the Human Prostate Cancer Antigen 3 ELISA KIT from HANGZHOU EASTBIOPHARM CO., LTD., located in Hangzhou, China.

Data collection

Following the acquisition of the code of ethics from Isfahan University of Medical Sciences and obtaining informed consent from the patients through interviews, the participants in each group were randomly selected based on the inclusion criteria. The purpose and details of the study were explained to the patients, and their demographic information, including age, was collected along with the main variables of the study, namely serum total PSA, free PSA, and PCA3 levels, which were measured in both groups.

The study aimed to examine the average values of the three variables (serum total PSA, free PSA, and PCA3) in both the case and control groups. Additionally, the sensitivity and specificity of these variables in diagnosing prostate cancer were evaluated.

Data analysis

The statistical analysis was done by SPSS version 26.0 software. Quantitative data are reported as mean and standard deviation and qualitative data are reported as percentages and frequency. We used independent T test to compare data between two groups. The normality of the data was confirmed using the Kolmogorov-Smirnov test. In order to determine the sensitivity and specificity in the present study, the Roc curve was used. Significance level in the present study is considered less than 0.05.

Ethical issues

The research adhered to the principles outlined in the Declaration of Helsinki, which provides ethical guidelines for medical research involving human subjects. The Ethics Committee of Isfahan University of Medical Sciences approved the study, and all study protocols were reviewed and approved by the institutional ethical committee at Isfahan University of Medical Sciences, with the ethical code: IR.MUI.MED.REC.1399.022. Prior to any intervention, written informed consent was obtained from all participants, ensuring their voluntary participation and understanding of the study's objectives and procedures. It is important to note that this study was derived from an M.D. dissertation conducted at Isfahan University of Medical Sciences.

Results

Study population

A total of 50 patients with suspected prostate cancer were included in the case group, while the control group also consisted of 50 individuals. The two groups were carefully matched in terms of gender, age, and body mass index (BMI). Statistical analysis revealed no significant differences in mean age and BMI between the two groups. Furthermore, the distribution of gender in the study groups did not show any significant differences. The age range of participants in the study spanned from 50 to 90 years.

PSA and PCA3 levels

Table 1 presents the mean values of Free PSA levels, Total PSA levels, and PCA3 levels in the case and control groups. The results of **Table 1** indicate that the mean of Free PSA levels in the control group is significantly higher than that in the case group ($P<0.05$). Additionally, the mean of Total PSA levels in the case group is significantly higher than that in the control group

Table 2. Determination of sensitivity and specificity and cut-off in the diagnosis of people with prostate cancer

Variables	AUC	*P*-value	Cut-off	Sensitivity	Specificity
Total PSA levels	0.95	0.001	≤3.14	93%	82%
The PCA3 levels	0.62	0.04	≤3.5	70%	72%

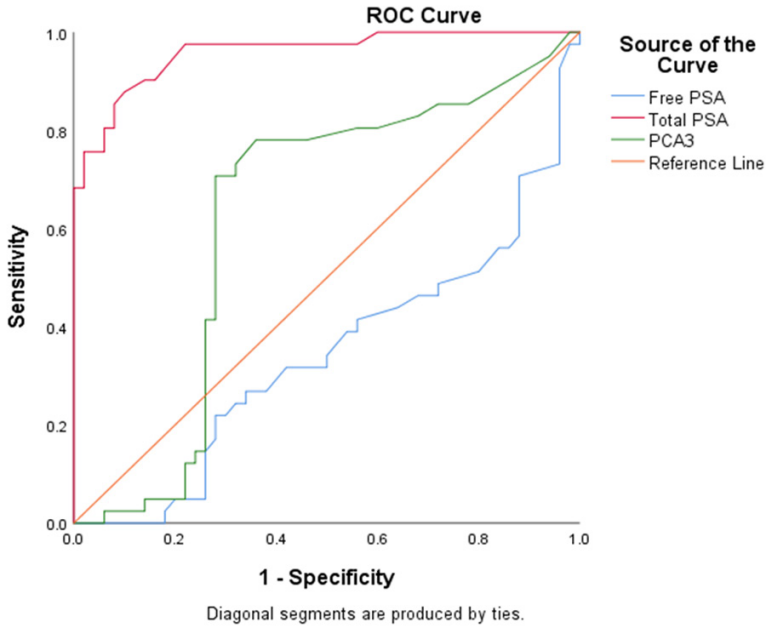

Figure 1. ROC curve diagram to determine the diagnosis and the sensitivity and specificity of the tests used in the present study.

(P<0.05). However, no significant difference was observed in the mean of the PCA3 levels between the case and control groups.

Sensitivity and specificity

Table 2 and **Figure 1** display the sensitivity and specificity of the two variables that exhibited significant differences in diagnosing individuals with and without prostate cancer. According to the results presented in **Table 2**, the variable Total PSA with a cut-off value of ≤3.14 demonstrated a sensitivity of 93% and specificity of 82%, indicating the highest diagnostic accuracy in distinguishing between prostate cancer and healthy individuals. Additionally, the value of PCA3 with a cut-off value of ≤3.5 yielded a sensitivity and specificity of 70% and 72%, respectively.

Discussion

In this study, we examined three main markers commonly used for prostate disease screening. The results revealed that the Total PSA test exhibited higher diagnostic accuracy compared to the other two tests. This test correctly diagnosed 90% of individuals with cancer (sensitivity) and 80% of healthy individuals (specificity). However, the PCA3 test also demonstrated acceptable diagnostic capability, accurately identifying 70% of healthy individuals and those with cancer.

Regarding the mean values, the control group showed significantly higher levels of Free PSA compared to the case group (P<0.05). Conversely, the case group exhibited significantly higher levels of Total PSA compared to the control group (P<0.05). However, there was no significant difference observed in the mean levels of PCA3 between the case and control groups. Another study conducted by Yazdani et al. examined serum and urinary PCA3 levels in patients with BPH and prostate cancer in a cross-sectional study involving 90 individuals (38 with prostate cancer and 52 with BPH). The results of their study indicated significantly lower levels of PCA3 and total PSA in individuals with cancer compared to those with hyperplasia. If you have any further questions or if there's anything else I can assist you with, please let me know [15].

The study conducted by Shafi et al. [16] involved 103 cases, among which 15 cases were diagnosed with malignancy. Six of these cases showed abnormal PSA levels, while six had abnormal findings in the digital rectal examination (DRE). Three cases exhibited abnormalities in both tests. Yazdani et al. [15] reported on 85 cases of prostate cancer and found that the free/total PSA ratio and serum total PSA were associated with the Gleason score intensity index, indicating a relationship between these markers and the aggressiveness of the cancer.

In a study by Wei et al. [17] which included 859 male subjects with a mean age of 62 years, elevated levels of PCA3 were found to be asso-

ciated with malignancy. Similarly, our study established a significant difference in serum PCA3 levels between individuals with and without suspected prostate cancer. Luca et al. [18] in a study involving 274 cases, demonstrated the significant roles of both PSA and PCA3 in the diagnosis of prostate malignancy, consistent with the findings of our study.

Cao et al. [19] reported high sensitivity and specificity for PCA3, confirming its utility as a diagnostic marker, which aligns with the findings of our study. Additionally, Saini et al. [20] mentioned in their review study that novel biomarkers, including PCA3, are being developed as potential alternatives to serum PSA levels, further supporting the diagnostic role of PCA3 observed in our study. Birnbaum et al. [21] reported that PCA3 exhibited good diagnostic value and significantly reduced false positive results, which is consistent with our study findings. Huang et al. [22] demonstrated that PCA3 can enhance the sensitivity and specificity of screening tests for prostate cancer, further supporting our study results.

Asgari et al. conducted a similar study in Iran to investigate the serum levels of total PSA and urine levels of PCA3 in patients with benign prostatic hyperplasia (BPH) and prostate cancer. The results of their study indicated that the mean levels of PCA3 and total PSA were significantly higher in patients with prostate cancer compared to patients with BPH ($P<0.05$) [23].

However, it is important to note that the study had certain limitations. One limitation was the inability to assess other potential confounding variables that could influence the results. Variables such as age, body mass index, and other relevant factors were not collected or considered in the study. Additionally, the financial support for the research was obtained solely from a university grant, potentially limiting the extent of resources available for the study.

Conclusion

In summary, the findings of the study suggest that the total PSA test has a higher accuracy in detecting prostate cancer in individuals suspected of having the disease compared to healthy individuals. It is considered a useful screening marker, and the study recommends the simultaneous measurement of total PSA alongside other biomarkers. However, to obtain more specific and reliable results, future studies with larger sample populations and consideration of other potential confounding variables are needed. These efforts would contribute to better decision-making regarding the use of PCA3 as a diagnostic tool.

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Disclosure of conflict of interest

None.

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