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The importance of plasma arginine level and its downstream metabolites in diagnosing prostate cancer

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Abstract

Purpose There is still no certain threshold value of prostate-specific antigen (PSA) for prostate cancer diagnosis. We aimed to investigate the predictive value of arginine and its metabolites for diagnosing prostate cancer in patients with PSA 4–10 ng/ml and evaluate their usefulness as prognostic tumor markers.

Methods Seventy-eight patients with a mean age of 64.50 ± 5.49 years were included in our prospective observational study between November 2016 and March 2017. They were divided into two equal groups according to the pathologic results of prostate biopsy (benign vs. malignant). Plasma arginine and ornithine levels were analyzed before biopsy by liquid chromatography–tandem mass spectrometry. ELISA was used for analyzing urinary diacetylspermine.

Results In PSA-adjusted analysis, the malignant group had lower plasma arginine levels ($p = 0.021$) and arginine to ornithine ratio (AOR) ($p = 0.010$), but higher plasma ornithine levels ($p = 0.012$) and urinary diacetylspermine levels ($p < 0.001$) as compared with the benign group. While arginine ($r = -0.628$, $p < 0.001$) and AOR ($r = -0.714$, $p < 0.001$) were negatively correlated with D'Amico clinical classification ($p < 0.001$), ornithine ($r = 0.659$, $p < 0.001$) and diacetylspermine ($r = 0.710$, $p < 0.001$) were found to be positively correlated ($p < 0.001$). In multivariate analysis, ornithine [OR 3.264, 95% CI (1.045–10.196), $p = 0.042$] and diacetylspermine [OR 6.982, 95% CI (2.403–20.290), $p < 0.001$] were found to be more significant in detection of prostate cancer.

Conclusion Plasma arginine, ornithine, AOR and urinary diacetylspermine levels may be used as molecular markers to predict prostate biopsy outcomes in patients with PSA 4–10 ng/ml. But according to our results, the use of ornithine and diacethylspermine prior to biopsy seems to be the most cost-effective diagnostic strategy.

Keywords Arginine · Diacetylspermin · Ornithine · Prostate biopsy · Prostate cancer · Tumor marker

Introduction

Prostate cancer is the most common non-cutaneous malignancy in males worldwide, and the fifth most common malignancy in the general population. When cancer-related deaths are considered, it is the second most common cause of death after lung cancer [1]. The risk of having prostate cancer for a man during his life is 16.72%; mortality due to prostate cancer is 2.57% [2]. Because it is a major public health problem, early recognition of prostate cancer is of paramount importance.

Although a prostate-specific antigen (PSA) value of ≥ 4 ng/ml is recommended for biopsy, there is still no certain threshold value of PSA for accurate cancer diagnosis [2]. In addition, since PSA is not specific to prostate cancer, there may be an increase in many benign prostatic diseases (benign prostate hyperplasia, prostatitis, urinary retension,

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etc.). In biopsies taken from men with a PSA value of 4–10 ng/ml, the cancer detection rate is between 20 and 25% [3, 4]. This leads to an unnecessary invasive procedure in a significant proportion of patients. Molecules and markers that may have a higher positive predictive value than PSA in diagnosis of prostate cancer have been investigated to prevent unnecessary biopsy [5].

In recent years, significant findings have been obtained in the literature that arginine, ornithine and polyamines play an important role in cancer development [6]. In particular, urinary diacetylspermine level, a polyamine derivative, has been found to be a tumor marker with high sensitivity in breast cancer, colorectal cancer and non-small cell lung cancer [7]. Moreover, it has been shown that urinary polyamines are significantly elevated in patients with various cancers, even in early stages [8].

Based on these findings, we aimed to assess whether arginine and its downstream molecules have a better predictive value than PSA to detect prostate cancer in patients with a PSA value of 4–10 ng/ml.

Materials and methods

Patients and study design

A prospective observational study was designed after approval of the local ethics committee (protocol number: 2016-10/01) and written informed patient consent between November 2016 and March 2017. Patients with age between 50 and 75 years who underwent transrectal ultrasound-guided prostate biopsy were included in our study. All patients were asymptomatic with a normal digital rectal examination and a PSA level between 4 and 10 ng/ml. The criteria for exclusion from our study are listed below:

- Patients who had a history of any malignancy before prostate biopsy
- Patients whose pathologic evaluation of biopsy specimens was not clear (high grade prostatic intraepithelial neoplasia or atypical small acinar proliferation)
- Conditions that may affect urinary diacetylspermine levels and PSA levels (patients with ulcerative colitis, systemic lupus erythematosus, active urinary infection; patients whose hemoglobin, direct bilirubin or indirect bilirubin levels were abnormal; those who used foods or medicines containing ascorbic acid more than the daily requirement)

Selection of patients

Blood and urine samples were taken from all volunteer patients before the biopsy without any known pathological results. Patients were divided into two groups after prostate biopsy results. The first group consisted of patients with prostate adenocarcinoma ($n = 47$) and the second group consisted of patients with benign pathological diagnosis ($n = 129$) (benign prostatic hyperplasia, chronic prostatitis, tumor-free prostate tissue, etc.). 78 of the 176 patients were selected following age and PSA-adjusted analysis. The flowchart of the study population and randomization of each group is shown in Fig. 1.

Storage and transfer conditions of blood and urine samples

A tube of fasting blood sample and the urine sample was taken between 8.00 and 9.00 a.m. on the day of prostate biopsy. The blood samples were separated into the plasma section after being centrifuged in the biochemistry laboratory as soon as the samples were taken. The plasma portion and the urine samples were stored at − 20 °C in the laboratory. After biopsy outcomes were determined, 78 of a total of 176 patients were included in the study following PSA-adjusted analysis and randomization. Their samples were sent to the Central Biochemistry Laboratory for biochemical analysis, providing transfer conditions of − 20 °C.

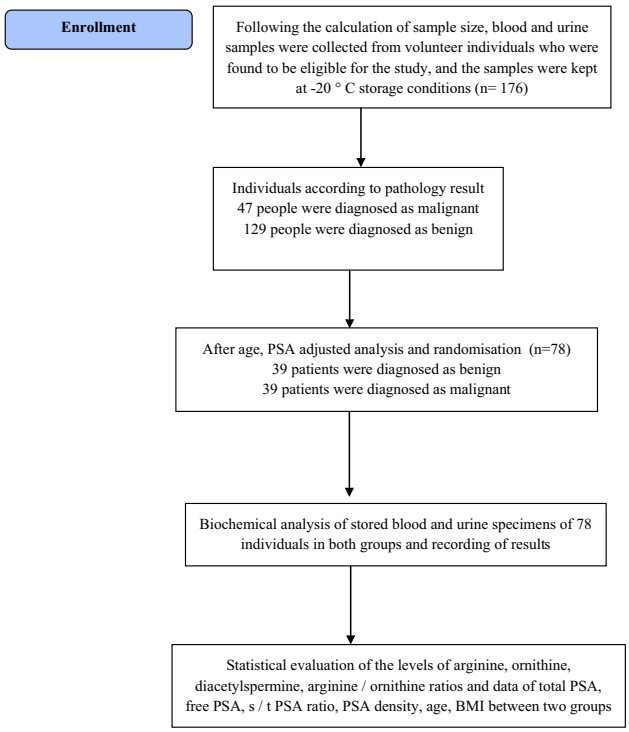

Fig. 1 Flowchart of the patients

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis of blood samples

Analysis of arginine and ornithine was performed using an LC–MS/MS chromatographic method (Thermo Fisher Scientific-168 Third Avenue, Waltham, MA 02451). Mass spectrometry is an analytical device that performs separation of charged particles moving in magnetic or electrical fields according to mass/charge ratios. It separates ions according to the mass/load ratios. The plasma samples collected from the patients were adjusted to room temperature. 20 µl of internal standard mix solution was combined with 20 µl of plasma and then mixed in a vortex mixer for 5 s. 400 µl of precipitation reagent was added and mixed in the vortex mixer for 10 s. This was centrifuged for 5 min at 14,000 rpm (revolutions per minute). The clear supernatant was transferred to a vial. In this way, the amounts of L-arginine hydrochloride and L-ornithine monochloride in plasma were measured. Arginine to ornithine ratio (AOR) was also calculated.

Determination of urinary diacetylspermine by an ELISA method

Urinary diacetylspermine analysis was performed using a diacetylspermine ELISA Kit (Abnova- No. 326-8, Sec. 4, Zhongzheng Rd. Zhongli Dist., Taoyuan City 320 Taiwan). Frozen urine samples were adjusted to room temperature. After centrifugation for 10 min at 1500 rpm, the supernatant was diluted 1:4 with distilled deionized water. Six different concentrations of standard solutions were prepared. 70 µl of anti-diacetylspermine antibody solution, 70 µl of six standards and 70 µl of prepared urinary sample were mixed in secondary tubes (preincubation solution) and incubated at room temperature for 1 h. 50 µl of preincubation solution was added to each well and re-incubated for 1 h. After incubation, the wells were washed with 300 µl of wash solution three times. After incubation, the washing process was repeated and the absorbance at a wavelength of 490 nm was measured using a Model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). Diacetylspermine concentration (nM) was calculated by plotting a standard curve and adjusted diacetylspermine concentration (nmol/gr.cre) based on urine creatinine (mg/dl) was used as the actual measurement

$$(\text{nmol/gr.cre}) = \frac{\text{Diacetylspermine concentration (nM)}}{\text{Creatinine concentration (mg/dl)}} \times 100.$$

Collection of other data and evaluation of biopsy outcomes

Data of age, body mass index (BMI), total PSA (T-PSA), free PSA (F-PSA) and free/total PSA (F/T-PSA) were recorded before biopsy. The prostate volumes and PSA density were calculated. Prostate biopsy was performed using transrectal ultrasonography following periprostatic nerve block, taking 12 core samples as standard technique by the same urologist. Patients with prostate adenocarcinoma were classified according to the International Society of Urological Pathology (ISUP) 2014 grades and D'Amico risk classification [9].

Statistical analysis

G*Power (G*Power Ver. 3.0.10, Franz Faul, University Kiel, Germany, http://www.psycho.uni-duesseldorf.de/aap/projects/gpower) package program was used for determining sample size. The sample size was calculated as at least 39 individuals in each group to obtain a test power of 80.4%, confidence interval of 90%, Type I error (alpha) of 0.05 and effect size of 0.7. Shapiro–Wilk tests were used for the evaluation of normality. Independent samples *t* test was used for detecting the differences between two groups of normally distributed variables, and Mann–Whitney *U* test was performed in non-normal distribution. The relationship between variables was assessed by Pearson Correlation test. Receiver operating characteristic (ROC) curve analysis was performed to determine cutoff values for arginine and its downstream molecules. Binary logistic regression analysis was used to determine the predictive factors for prostate cancer diagnosis. A p value of < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics 21 (IBM, Armonk, NY, USA).

Results

Patient characteristics

The present study consisted of 39 male patients who were diagnosed as benign (mean age 63.79 ± 5.27 years, range 54–74 years) and 39 male patients who were diagnosed as malignant (mean age 65.21 ± 5.68 years, range 56–74 years). There were no significant differences between groups regarding age and body mass index. Total PSA, free PSA, F/T-PSA, PSAD and prostate volumes were also compared between the groups. No significant differences were found in terms of these parameters. The results are shown in Table 1. Plasma arginine levels and the value of AOR were significantly lower in the malignant group. Conversely, plasma ornithine and urinary

Table 1 Patient demographics and clinical data

Variables	Benign ($n=39$)	Malignant ($n=39$)	Total ($n=78$)	p
Age (years)	63.79 ± 5.27	65.21 ± 5.68	64.50 ± 5.49	0.259[†]
BMI (kg/m^2)	27.56 (25.29–30.10)	27.51 (25.85–29.19)	27.51 (25.50–29.96)	0.441[§]
Arginine (μmol/L)	92.86 ± 33.00	74.54 ± 35.42	83.69 ± 35.23	0.021*[†]
Ornithine (μmol/L)	118.16 ± 20.18	136.70 ± 40.29	127.43 ± 32.99	0.012*[†]
Arginine to ornithine ratio	0.75 (0.31–1.45)	0.54 (0.10–2.27)	0.70 (0.42–0.93)	0.010*[§]
Diacetylspermine (nmol/g.cre)	104.17 (62.71–127.63)	123.68 (70.73–208.78)	113.99 (97.05–126.38)	< 0.001*[§]
Total PSA (ng/ml)	5.83 (4.30–9.00)	7.41 (4.01–10.32)	6.00 (4.83–8.44)	0.058[§]
Free PSA (ng/ml)	1.33 ± 0.59	1.39 ± 0.74	1.36 ± 0.66	0.661[†]
Prostate volume (ml)	45 (35–57)	42 (33–58)	45 (34–57)	0.838[§]
Free/total PSA ratio	0.22 (0.03–0.42)	0.20 (0.03–0.45)	0.21 (0.15–0.25)	0.287[§]
PSAD (ng/ml^2)	0.12 (0.05–0.38)	0.16 (0.04–0.57)	0.15 (0.10–0.19)	0.267[§]

Data expressed as mean ± standard deviation or median (25–75 percentiles)

BMI body mass index, *PSA* prostate-specific antigen, *PSAD* prostate-specific antigen density

*$p < 0.05$; asterisk (*) indicates statistical significance

[†]Independent sample *t* test

[§]Mann–Whitney *U* test

Table 2 Pathological features of the malignant group

D'Amico risk classification (n, %)	
Low risk[a]	16 (41.1)
Intermediate risk	9 (23.1)
High risk	14 (35.8)
2014 ISUP grade (n, %)	
1	25 (64.1)
2	5 (12.8)
3	4 (10.2)
4	2 (5.1)
5	3 (7.8)
Clinical TNM stage (n, %)	
cT1c	13 (33.3)
cT2a	7 (17.9)
cT2b	7 (17.9)
cT2c	12 (30.9)

The criteria of clinically significant prostate cancer are the following: clinical stage ≥ T2, Gleason score ≥ 7, > 2 positive cores and the percentage of tumor in each positive core > 50% on biopsy. Clinically insignificant cancer does not have any of these criteria

ISUP International Society of Urological Pathology, *TNM* tumor-node-metastasis

[a]Five patients had clinically insignificant prostate cancer

diacetylspermine levels were significantly higher in the malignant group (Table 1). Pathological features of the malignant group are also shown in Table 2.

Correlation between clinicopathological tumor characteristics and arginine metabolites

In patients who had higher pathological 2014 ISUP grade and D'Amico risk group, it was observed that plasma arginine level and AOR significantly decreased while ornithine and diacetylspermine levels significantly increased (Table 3). We also observed a weak positive relationship between T-PSA and ornithine (r: 0.269, $p = 0.011$), diacethylspermine (r: 0.336, $p = 0.001$). On the other hand, there was a weak negative relationship between T-PSA and AOR (r: −0.230, $p = 0.031$).

ROC and regression analysis of metabolites separating malignant and benign groups

The ROC curve was drawn for plasma arginine, ornithine, AOR and urinary diacetylspermine. The area under curve (AUC) of these parameters was calculated as 0.653 ($p = 0.020$), 0.674 ($p = 0.008$), 0.669 ($p = 0.010$) and 0.779 ($p < 0.001$), respectively. The cutoff values, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are shown in Table 4. In univariate analysis, arginine [OR 3.053, 95% CI (1.175–7.928), $p = 0.022$], ornithine [OR 4.812, 95% CI (1.716–13.493), $p = 0.003$], AOR [OR 3.509, 95% CI (1.324–9.295), $p = 0.012$] and diacetylspermine [OR 8.719, 95% CI (3.104–21.488), $p < 0.001$] were found as independent factors to predict prostate cancer. But according to multivariate analysis, ornithine [OR 3.264, 95% CI (1.045–10.196), $p = 0.042$] and diacetylspermine [OR 6.982, 95% CI (2.403–20.290), $p < 0.001$] were statistically more significant (Table 5). According to

Table 3 The relationship between biomarkers and staging systems

	Pathological ISUP grade ρ	p	D'Amico risk group ρ	p
Arginine (µmol/L)	−0.533	<0.001*	−0.628	<0.001*
Ornithine (µmol/L)	0.661	<0.001*	0.659	<0.001*
AOR	−0.658	<0.001*	−0.714	<0.001*
Diacetylspermine (nmol/g.cre)	0.387	0.009*	0.710	<0.001*

Pearson test was performed for correlation analysis. ρ, (p value)

ISUP International Society of Urological Pathology, *AOR* arginine to ornithine ratio

*$p < 0.05$ asteriks (*) indicates statistical significance

Table 4 Cut-off values of additional parameters in predicting prostate cancer

	Arginine (µmol/L)	Ornithine (µmol/L)	AOR	Diacetylspermine (nmol/g.cre)
Cut-off value	67.18	135.67	0.549	117.26
Sensitivity (%)	51.3	51.3	51.3	69.2
Specificity (%)	74.4	82.1	76.9	79.5
PPV (%)	66.7	74	68.9	77.1
NPV (%)	60.4	62.7	61.2	72.1
AUC	0.653	0.674	0.669	0.779
p	0.020*	0.008*	0.010*	<0.001*

AOR arginine to ornithine ratio, *PPV* positive predictive value, *NPV* negative predictive value, *AUC* area under curve

*$p < 0.05$ asteriks (*) indicates statistical significance

the multivariate analysis, when we performed a model that includes all four new parameters to predict prostate cancer, the values of sensitivity, specificity, PPV, NPV and AUC were found to be 74.3%, 79.5%, 78.3%, 75.6%, 0.849, respectively. These values were higher than the values for each individual parameter. The cost-effectivity analysis is shown in Fig. 2. According to our results, in addition to T-PSA, the use of ornithine and diacethylspermine prior to biopsy seems to be the most cost-effective diagnostic strategy (Fig. 2).

Discussion

Prostate biopsy is an invasive procedure in males for prostate cancer detection and can have several complications, including sepsis, rectal bleeding, hematuria, hematospermia, fever, urinary tract infection and rectal pain. It may also require general anesthesia. Prostate-specific antigen may be affected by age, race, prostate volume and urinary infection. There is still no certain cutoff value (9). When the T-PSA value is taken as 4.0 ng/mL, the sensitivity for detecting prostate cancer is 20% and the specificity is between 60 and 70% [4, 9]. This result leads to a deficiency in diagnosis, prognosis and follow-up. Prostate-specific antigen derivatives (free PSA percentage, PSA density, PSA velocity, PSA doubling time, PSA adjusted for transitional zone volume) were determined to increase the sensitivity and specificity in the diagnosis. However, these PSA parameters do not have a threshold value and they are also influenced by many factors, so their reliability is low [9, 10]. New markers have been investigated to reduce the frequency of unnecessary biopsies performed to detect clinically significant prostate cancer [11–13]. Additionally, to avoid unnecessary biopsies, current studies recommend a risk calculator, imaging, additional serum or urine-based tests such as PSA derivatives, prostate cancer antigen 3 (PCA3), prostate health index (PHI) and 4 K-panel before

Table 5 Predictive factors for prostate cancer

Cut-off values	Univariate model				Multivariate model			
	OR	95% Confidence interval		p	OR	95% Confidence interval		p
		Lower	Upper			Lower	Upper	
Arginine (µmol/L) (≤67.18)	3.053	1.175	7.928	0.022*				0.517
Ornithine (µmol/L) (≥135.67)	4.812	1.716	13.493	0.003*	3.264	1.045	10.196	0.042*
Arginine to ornithine ratio (≤0.549)	3.509	1.324	9.295	0.012*				0.850
Diacetylspermine (nmol/g.cre) (≥117.46)	8.719	3.104	21.488	<0.001*	6.982	2.403	20.290	<0.001*

Logistic regression analysis OR = odds ratio

*$p < 0.05$ asteriks (*) indicates statistical significance

Parameters	Cost for one sample (EUR)*
Material cost for biopsy	26.41
Personnel cost for biopsy	6.56
Coagulation test	5.26
Pathological analysis	12.49
Cost for antibiotic prophylaxis	9.22
Total cost for biopsy procedure	**59.94**
T-PSA	1.66
F-PSA	2.61
Arginine	0.56
Ornithine	0.56
Diacethylspermine	13.99

*The average unit costs for 2019 in Turkey are used to determine the costs for each parameters.

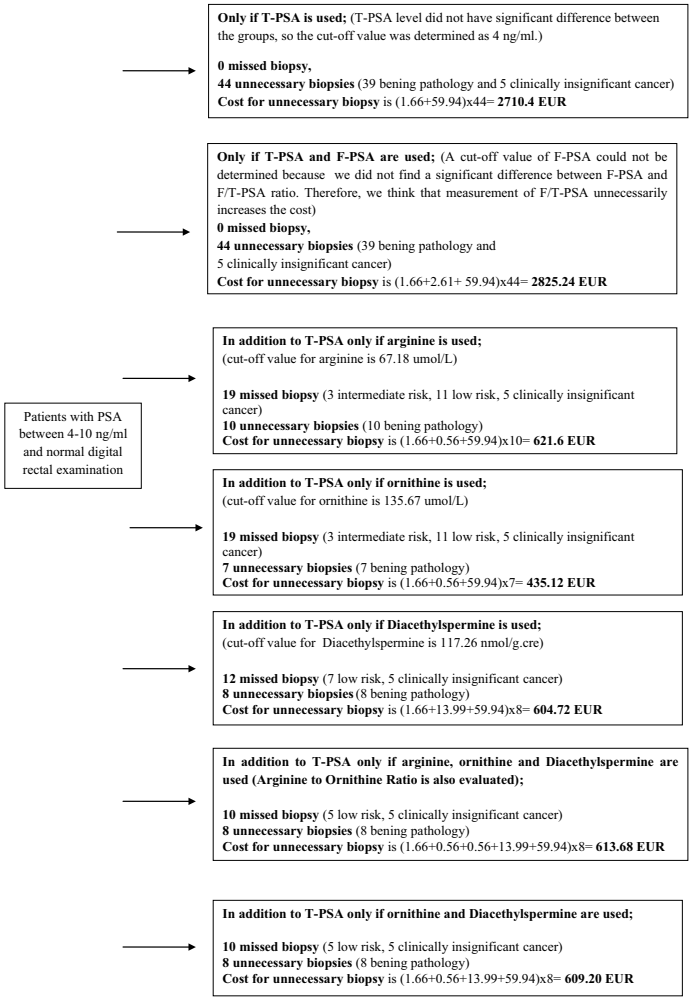

Fig. 2 Decision tree of different diagnostic strategies to detect prostate cancer and the costs of unnecessary biopsies for each strategy

performing prostate biopsy in asymptomatic men with a normal digital rectal examination and PSA level between 2 and 10 ng/mL [9–14]. Studies on the relationship between prostate cancer and its biological metabolism have generated great interest in the last decades [9].

Arginine, which plays an important role in cancer development and progression, is a semi-essential amino acid [6]. It is required for basic biological processes such as protein synthesis and production of polyamines (putrescine, spermine, spermidine) in both normal and malignant cells [15].

Arginine is converted to ornithine by arginase 1. Ornithine is transformed into polyamines via a series of catabolic reactions. Polyamines are involved in several biological pathways, such as gene expression, translation, cell proliferation, membrane stabilization and apoptosis. They play important roles in various events such as tumor growth, invasion and metastasis development [16]. Diacetylspermine is a small polyamine derivative that constitutes 0.46% of all polyamines in the urine of healthy individuals. In particular, it is argued that diacetylspermine, which is the last product of arginine catabolism, can be used as a tumor marker with a very low false-negative rate and high sensitivity in breast cancer, colorectal cancer and non-small cell lung cancer [6, 17, 18]. In addition, increases in urinary diacetylspermine levels have been reported as a poor prognostic factor in these cancers [6, 19, 20].

Derezinski et al. [21] stated that arginine and branched-chain amino acid metabolic pathways can be valuable markers for diagnosing prostate cancer. Serum arginine level was found to be lower in patients with prostate cancer than a control group. In this context, depletion of arginine by recombinant human arginase was considered as a novel anti-cancer strategy in prostate cancer by Hsueh et al. [22]. But they did not mention any cutoff value for arginine and its downstream metabolites were not investigated. Compatible with their results, we found significantly lower serum arginine level in the malignant group. Additionally, we calculated a cutoff value for arginine as 67.18 µmol/L.

Satoh et al. [23] investigated diacetylspermine levels in prostate cancer patients with and without bone metastases. Diacetylspermine levels were found to be higher in metastatic patients and this increase was associated with higher PSA levels. This was the first study to evaluate the importance of diacetylspermine in prostate cancer with bone metastases. In our study, arginine and its metabolism molecules were also investigated in patients having benign or malignant prostate tissue. Although no significant differences were found between our homogeneous groups in terms of T-PSA, decreased arginine level and increased ornithine and diacetylspermine levels were significant in the malignant group. We calculated a cutoff value of 117.46 nmol/g.cre for diacetylspermine. This was the most significant marker in the malignant group. Above this level, diacetylspermine had a 6.982 times greater risk of prostate cancer detection. To our knowledge, this is the first study to evaluate the presence of the arginine–ornithine–diacetylspermine metabolic pathway specifically in prostate cancer cases with PSA values between 4 and 10 ng/ml.

In the literature, the importance of arginine and downstream metabolites was emphasized in breast, colorectal and non-small cell lung cancers [6, 8, 24]. Hu et al. [6] observed significant declines in plasma arginine level and AOR in patients with breast cancer. Conversely, significant increases in plasma ornithine level and urinary diacetylspermine concentration were observed in these patients. In accordance with their results, we observed similar changes in the metabolic pathway of arginine in patients with prostate cancer. As we can see, these markers are not specific to prostate cancer. However, they are determinative in predicting different cancers using different cutoff values.

The value of any diagnostic test is a balance between the sensitivity and specificity. Specificity refers to being truly disease free. The closer the specificity is to 100%, the more likely the diagnostic test may prevent patients from obtaining unnecessary treatments. An optimal threshold value is a compromise between sensitivity and specificity and is determined based on the intended use. High sensitivity is more important in a clinical cohort, while high specificity is more important in a population-based screening [25]. In our study, we determined the threshold values with higher specificity values without reducing the sensitivity below 50%. The optimal threshold value also depends on what risk of missing prostate cancer is clinically acceptable. The criteria of clinically significant prostate cancer are the following: clinical stage $\geq$ T2, Gleason score $\geq 7, > 2$ positive cores and the percentage of tumor in each positive core $> 50\%$ on biopsy [9]. The clinically significant prostate cancer rate in our malignant group was 87.2%. In our study, the number of patients with clinically insignificant prostate cancer was quite small, so we could not compare the levels of arginine metabolites between clinically significant and insignificant patient groups. In studies with an adequate number of patients, if there is a significant difference between the levels of these metabolites and the new predictive values can be determined, unnecessary biopsies applied to patients with clinically insignificant prostate cancer may be further decreased.

When the new-age biomarkers for prostate cancer were investigated, the range of specificity for PCA3 was 72–79% [26]. Furthermore, the specificity of PHI was 32% [27]. In a multivariable model, Vedder et al. [28] did not find a statistically significant difference between PCA3 (AUC 0.73) and the 4 K-panel (AUC 0.71; $p = 0.18$). Russo et al. [29] stated that both PHI and the 4 K panel had good diagnostic accuracy in detecting prostate cancer. A sensitivity of 89%, specificity of 34% and AUC of 0.76 were determined for PHI, whereas the sensitivity was 74%, specificity was 60% and AUC was 0.72 for the 4 K panel [29]. However, a 100% diagnostic test for the detection of cancer is still not available. It is seen that the specificity values of the biomarkers we use, particularly diacetylspermine, are higher than the markers mentioned above. The PPV, NPV and AUC of diacetylspermine are even better than these markers reported in the literature. As a result, we anticipate that we will be able to prevent patients having unnecessary biopsies and complications that may arise following biopsy.

Our first limitation was the small number of patients. Previous studies of the arginine metabolic pathway investigated in other tumors besides prostate also had small numbers of participants. Our sample size based on statistical power analysis was larger than the other similar studies mentioned above. The results obtained from new prospective studies with larger patient numbers may allow us to make more accurate inferences on this subject.

Secondly, we had no group that included healthy volunteers, unlike other studies in the literature. Although it is possible to diagnose breast, colorectal or lung cancer by physical examination and imaging methods, there is no definite diagnostic method that can accept a person as a healthy individual, carrying no prostate cancer. There is also no definite cutoff value for PSA to determine patients who will undergo prostate biopsy. We also cannot ignore the fact that malignant areas in prostate tissue may have been missed even in individuals who have undergone biopsy and have no malignancy in pathologic evaluation. Moreover, in the literature, cancer detection rates for PSA ≤ 0.5/0.6–1.0/1.1–2.0/2.1–3.0/3.1–4.0 ng/ml are stated as 6.6%, 10.1%, 17.0%, 23.9% and 26.9%, respectively [30]. It is not methodologically correct to incorporate a male into a healthy volunteer group only based on PSA value, since it is not possible to precisely detect or exclude cancer by radiological methods, even at the lowest PSA values. Nevertheless, we can express this situation as another limitation of our study.

Conclusion

In conclusion, arginine and its downstream molecules have a better predictive value than PSA in detecting prostate cancer. As a consequence, these molecules can be used as molecular markers to determine whether prostate biopsy should be performed in patients with PSA of 4–10 ng/ml. But the use of ornithine and diacethylspermine prior to biopsy seems to be the most cost-effective diagnostic strategy. Further research is required for the validation of our findings to detect clinically significant prostate cancer.

Author contributions IS: conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript. HB: conception and design, critical revision of the manuscript for important intellectual content. NB: analysis and interpretation of data, critical revision of the manuscript for important intellectual content. KM: biochemical analysis. OK: statistical analysis

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical approval for research involving human participants The study was approved by the local ethics committee (protocol number: 2016-10/01) at Health Science University Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital. All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent A formal written informed consent was obtained from all individual participants included in the study. The data of patients who did not consent were not used.

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