DOI: 10.4274/jarem.galenos.2022.74046
J Acad Res Med 2022;12(2):42-8

Can Urine Sarcosine Predict the Prostate Biopsy Necessity in Patients with Total PSA Value Ranging Between 2.5-10 ng/mL?

Onur Fikri[1], Nilhan Nurlu[2], Mustafa Bahadır Can Balcı[1], Ali Eroğlu[3], Memduh Aydın[1], Arif Kalkanlı[1], Cem Tuğrul Gezmiş[1], Barış Nuhoğlu[4]

[1]University of Health Sciences Turkey, Taksim Training and Research Hospital, Clinic of Urology, Istanbul, Turkey
[2]University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital, Clinic of Clinical Biochemistry, Istanbul, Turkey
[3]İzmir Torbalı State Hospital, Clinic of Urology, Izmir, Turkey
[4]Biruni University Hospital, Clinic of Urology, Istanbul, Turkey

Cite this article as: Fikri O, Nurlu N, Balcı MBC, Eroğlu A, Aydın M, Kalkanlı A, Gezmiş CT, Nuhoğlu B. Can Urine Sarcosine Predict the Prostate Biopsy Necessity in Patients with Total PSA Value Ranging Between 2.5-10 ng/mL?. J Acad Res Med 2022;12(2):42-8

ABSTRACT

Objective: The most common oncologic disease in men is prostate cancer. There have been studies for alternative methods for early screening. Over the past years, the interest in sarcosine as a potential marker for prostate cancer has increased. We evaluated the predictability of prostate biopsy necessity by using urine sarcosine for prostate cancer examination during our study.

Methods: The study included 84 male patients aged between 45 and 79 in our hospital between 15.12.2013 and 15.03.2014. After the primary evaluation, standard 12 cores transrectal ultrasonography prostate biopsy was performed by the clinician to the appropriate patients with total prostate specific antigen (PSA) values ranging between 2.5-10 ng/mL. A sarcosine measurement with colorimetric and fluorometric principles was performed on patients' urine samples taken before the prostate biopsy, following the prostate massage.

Results: Statistically significant negative correlation in malignant group and positive correlation in benign group were found between percentage change in PSA values and fluorometric sarcosine measurements ($r=-0.418$; $p=0.042$; $p<0.05$ / $r=0.318$; $p=0.013$; $p<0.05$ respectively).

Conclusion: The correlation between percentage change in PSA values and fluorometric sarcosine measurements can be used in patients with a grey zone PSA (such as PI-RADS 2-3 and low level PSA patients) in order to avoid unnecessary biopsies.

Keywords: Prostate cancer, prostate biopsy, sarcosine, urine, biomarker, prostate cancer screening

ORCID IDs of the authors: O.F. 0000-0002-5731-4620; N.N. 0000-0002-0844-5050; M.B.C.B. 0000-0003-0395-1154; A.E. 0000-0002-5545-5892; M.A. 0000-0002-5851-8246; A.K. 0000-0001-6509-4720; C.T.G. 0000-0002-1634-4516; B.N. 0000-0002-8737-4050.

Corresponding Author/Sorumlu Yazar: Onur Fikri,
E-mail: runo.runo@gmail.com

Received Date/Geliş Tarihi: 06.04.2021 **Accepted Date/Kabul Tarihi:** 29.03.2022

J Acad Res Med 2022;12(2):42-8

INTRODUCTION

Although the normal total prostate specific antigen (PSA) rate according to the EAU guidelines is still a subject to clarify, the guidelines suggest that the normal levels for young men are <2-3 ng/mL and 10 ng/mL total PSA level is set for the prognostic categorization of prostate carcinoma. Remarkably, it is still not clarified at what age the early screening should be started and between what levels the total PSA should be. However, it's recommended that the total PSA level be checked after the age of 40, and the screening is unnecessary after the age of 70 (1). In clinical practice, management of patients with total PSA level ranging between 2.5-10 ng/mL differs. Although some clinics recommend a transrectal ultrasonographic biopsy (TRUS-biopsy) directly, some clinics postpone the decision and examine a new total PSA level after antibiotic treatment (2).

Among those patients who have undergone a TRUS-biopsy, some of the patients have benign results. Prostate biopsies are found to be painful and stressful for many patients. Many patients refuse the procedure. The group of patients with benign results are exposed to unnecessary invasive manipulation because of the failure of total PSA in the prediction of cancer. On the other hand, some of the patients who refuse the procedure are advantageous because of being exempt from unnecessary biopsies while some of them are disadvantageous because of unawareness of their malignancy. Setting off on a quest for finding an adequate solution to this problem is important. We believe that urologists should provide comfort to the patients while diagnosing with the correct indication and avoiding missing malignant cases among this group. In recent years, the number of studies about prostate cancer screening, diagnosis, and the prediction of progression with the levels of a molecule called sarcosine in urine and serum is increasing (3-8). In our research, we aimed to identify if the sarcosine level in urine would be beneficial in the decision of TRUS-biopsy in patients with PSA level ranging between 2.5-10 ng/mL.

METHODS

The population of the study consisted of male patients who were admitted to Taksim Training and Research Hospital Urology Clinic. This study was a prospective, analytical study aiming to determine the ability of urine sarcosine levels of patients with total PSA level of 2.5-10 ng/mL in the prediction of prostate biopsy necessity. Study was performed between 15.12.2013-15.03.2014 after the Taksim Training and Research Hospital Clinical Research Ethics Committee approval (decision no: 29, date: 04.12.2013). Male patients between the ages of 40 and 79 were included in the study. All patients gave their informed consent for participation. The exclusion criteria were as follows; previous malignancy diagnosis, lower urinary tract surgery in last six months, the presence of active urinary tract infection, symptoms of abnormal digital rectal examination (frozen pelvis, rectal malignancy, etc.), lack of the sarcosine dehydrogenase enzyme, and the presence of sarcosinemia disease. Total PSA (in the first application and one month after the initial application on the day of the biopsy), free PSA, urine examination, uroflowmetry, International Prostate Symptom score, complete blood count, urine sarcosine level, ultrasonography (USG) measurements (prostate volume, residue urine after urinate) were examined. The data were recorded with the patient study registration form. As a result of the primary evaluation, standard 12 cores transrectal USG (TOSHIBA Aplio300) prostate biopsy was performed by the same clinician on the appropriate patients. After differentiating the patients according to the prostate biopsy reports as benign-malignant, the data were evaluated. Statistical comparison was performed concerning patients' other parameters, demographic features, and urine sarcosine levels during the diagnostic process.

Sarcosine Determination

Following the prostate massage before biopsy (after providing enough prostate fluid to pass to urine), the urine samples of patients were kept in -40 °C until the study day. The urine samples were melted in room temperature on study day and centrifuged for 10 minutes in 2000 g. The supernatant was separated.

Sarcosine kit (Sarcosine Assay Kit, Abcam, ab65338) was kept in -20 °C for two months until the study day (Table 1).

The sarcosine tampons and probes of the kit were ready to use. The sarcosine Enzyme Mix was melted in 220 µl sarcosine tampon with the help of an automatic pipette. Sarcosine standard was mixed with 100 µl distillate water, and 100 nmol/µl sarcosine standard was acquired. Sarcosine enzyme kit and standard were aliquoted and prepared to be examined. According to the test method, 46 µl sarcosine tampon, 2 µl sarcosine enzyme, and 2 µl probe were required to be added to each well; a reaction mix pool was created after the calculations of the tampon, enzyme, and probe number necessary for both the control and patient groups and mixed slowly with confounder.

As 10 µl of ready sarcosine standard was mixed with 990 µl sarcosine tampon, 1 nmol/µl standard study solution was prepared. After that, to prepare a standard curve, the standard study solution was pipetted in 0, 2, 4, 6, 8 µl 5 sequenced well, and each well's volume was completed to 50 µl. Also, 1 and 6 µl control samples were prepared with sarcosine standard and completed to 50 µl after pipetting in 2 different wells. After the enumeration of 84 patients' urine samples between 1 and 84, samples were pipetted to 50 µl wells in order. For each well, 50 µl reaction mix was added, and the wells plate was mixed with confounder. After incubation for 1 hour in 37 °C, EX/Em=544/590 nm fluorometric and 540 nm colorimetric was read. Concentration unit was determined as nmol/µl or millimolar.

Study was performed with 84 male patients aged between 45-79 with a mean of 60.49±6.81 years between 15.12.2013-15.03.2014 dates in the Urology Clinic of Taksim Training and Research Hospital.

Statistical Analysis

J Acad Res Med 2022;12(2):42-8

The Number Cruncher Statistical System Statistical Software (Utah, USA) program was used for statistical analysis. While evaluating the study data, quantitative variables were shown with mean, standard deviation, median, minimum and maximum values, and qualitative variables were shown with descriptive statistical methods such as frequency and percentage. Shapiro-Wilks test and Box Plot charts were used to evaluate the conformity of the data to the normal distribution. Mann-Whitney U test for the evaluation of non-normally distributed variables according to two independent groups; Wilcoxon Signed-Rank test was used in the evaluation of dependent groups according to their follow-up. Fisher's Exact test was used for the comparison of the qualitative data. Spearman's correlation analysis was used for the evaluation of the relationships between the pre-biopsy PSA parameters and PSA parameters on the day of the biopsy in colorimetric and fluorometric sarcosine measurements. The significance was evaluated at the levels of $p<0.05$.

RESULTS

Pathology results were benign in 71.4% (n=60), while they were malignant in 28.6% (n=24) of the patients. Gleason score was observed to be 6 in 79.2% (n=19), while it was 7 in 20.8% (n=5) of the malignant patients. Prostatitis was present in 63.1% (n=53) while it was not observed in 36.9% (n=31) of the patients.

Pre-biopsy PSA values of the patients ranged between 2.57 ng/mL and 9.89 ng/mL and mean was 5.85±1.97 ng/mL; PSA values on the day of the biopsy ranged between 0.84 ng/mL and 13.31 ng/mL and mean was 5.73±2.23 ng/mL. Percentage change in PSA values ranged between -90.7 ng/mL and 50.57 ng/mL and mean was -0.86±21.94 ng/mL.

While colorimetric sarcosine measurements ranged between 0 and 1.21, and the mean value was 0.44±0.24; fluorometric sarcosine measurements ranged between 0 and 1.3, and the mean was 0.36±0.27.

Prostate volume ranged between 15 ccs and 160 ccs, and the mean was 49.01±27.80 ccs.

No statistically significant difference was determined between pre-biopsy PSA measurements and PSA measurements on the day of the biopsy according to pathology results ($p>0.05$).

In benign group, change in PSA measurements on the day of the biopsy in the direction of reduction compared to pre-biopsy PSA measurements wasn't statistically significant ($p=0.123$; $p>0.05$), in malignant group, average increase in PSA measurements on the day of the biopsy compared to pre-biopsy PSA measurements wasn't statistically significant ($p=0.338$; $p>0.05$) (Table 2).

A statistically significant difference was determined between percentage change in pre-biopsy and on the day of the biopsy PSA measurements according to pathology results ($p=0.050$; $p<0.05$). While the mean percentage change in the benign patients was determined to be -3.44±22.43, it was 5.58±19.63 in malignant patients (Figure 1).

No statistically significant difference was determined between colorimetric and fluorometric sarcosine measurements of the patients according to pathology result ($p>0.05$) (Table 3).

A statistically significant difference was determined between prostate volume of the patients according to pathology result ($p<0.05$) and prostate volume in the malignant group was less than the benign group.

A statistically significant difference was determined between the prevalence rates of prostatitis in the patients ($p<0.01$). Prevalence of prostatitis in the malignant group was significantly less than the patients of the benign group.

No statistically significant correlation was determined between pre-biopsy PSA values and colorimetric and fluorometric sarcosine measurements and between PSA values on the day of the biopsy and colorimetric and fluorometric sarcosine measurements of the benign cases ($p>0.05$). No statistically significant correlation was determined in the malignant group, either ($p>0.05$).

No statistically significant correlation was determined between percentage change in PSA values and colorimetric sarcosine measurements of the benign patients ($p>0.05$). A statistically

Table 1. Sarcosine kit content

Sarcosine tamp	25 mL
Sarcosine prob (DMSO, anhidrosis)	0.2 mL
Sarcosine enzyme mix (lyophilised)	1 vial
Sarcosine standard (10 µmol, lyophilized)	1 vial

Table 2. Assessment of PSA measurements according to pathology result

	Pathology		[a]p
	Benign (n=60)	Malignant (n=24)	
	Median (min-max)	Median (min-max)	
Pre-biopsy PSA	5.42 (2.7-9.9)	6.06 (2.6-9.5)	0.443
PSA on the day of the biopsy	5.537 (0.8-13.3)	6.09 (3.2-10.5)	0.080
[b]p	0.123	0.338	
Pre-biopsy - PSA on the day of the biopsy percentage change (%)	-4.19 (-90.7/47.34)	1.05 (-29.5/50.6)	0.050*

[a]Mann-Whitney U test, [b]Wilcoxon Signed-Rank test, *p<0.05, PSA: prostate specific antigen, min: minimum, max: maximum

J Acad Res Med 2022;12(2):42-8

significant positive correlation was found between percentage change in PSA values and fluorometric sarcosine measurements (fluorometric sarcosine value increased with the increase of percentage change in PSA values) at a level of 31.8% (r=0.318; p=0.013; p<0.05).

A statistically significant negative correlation was found between percentage change in PSA values and colorimetric sarcosine measurements (as the percentage change in PSA values increased, colorimetric sarcosine value decreased) at a level of 41.5% in malignant patients (r=-0.415; p=0.044; p<0.05). It was also found the same in fluorometric sarcosine measurements at a level of 41.8% in malignant patients (r=-0.418; p=0.042; p<0.05) (Table 4).

There was no statistically significant difference between pre-biopsy PSA measurements and PSA measurements on the day of the biopsy (p>0.05) and between percentage change in PSA values of the malignant patients according to Gleason scores (p>0.05).

There was no statistically significant difference between colorimetric and fluorometric sarcosine measurements (p>0.05) and between prostate volumes of the malignant patients according to Gleason scores (p>0.05).

There was also no statistically significant difference between the prevalence rates of prostatitis in malignant patients according to Gleason scores (p>0.05) (Table 5).

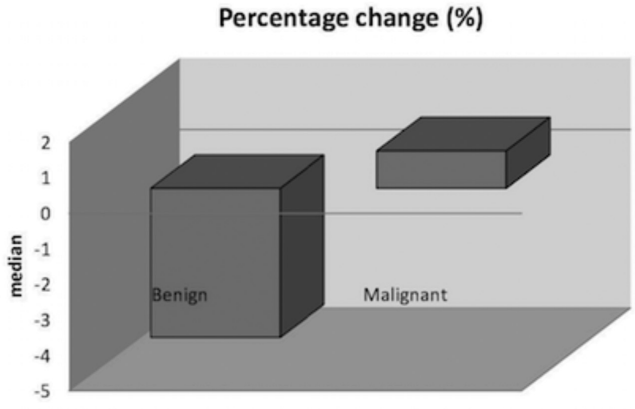

Figure 1. Percentage change (%) in pre-biopsy PSA measurements and PSA measurements on the day of the biopsy according to pathology results

PSA: prostate specific antigen

DISCUSSION

After the examinations of USA 2013 prostate cancer (PC) incidence-mortality data, it was seen that PC had the highest incidence (238.590) and second highest mortality (29.720) in all male cancer types (9). Due to those high ratios, early diagnosis of PC is vital. PSA is an organ-specific reagent. However, it has no specificity for disease or its degree. There are two main problems with PSA. The first problem is the great ratio of negative prostate biopsy results because PSA isn't specific for PC.

The second problem is that low level (0-20 ng/mL) PSA can't predict PC (3). Thus, it's thought that PSA is inadequate in PC screening and the researchers are looking for alternative reagents (10). Nowadays, the most used reagents in non-invasive disease are PCA-3 and annexin (3,4,11) in urine. New markers, such as urine alfa methyl alkyl CoA, which increases in adenocancer and high-grade intraepithelial neoplasia, are being discussed to be used in prediction of cancer progression (5,12-14). Another reagent is the ratio of fPSA/tPSA. It's reported that this ratio is especially effective in differentiating patients with PC and benign situations. Kallikrein 2, urokinase-type plasminogen activator/urokinase-type plasminogen activator receptor, IL-6/IL-6 receptor, pigment epithelium-derived factor, fibronectin 1, chromogranin A, ceruloplasmin are remarked as high potential bioreagents (15,16). In addition, sarcosine; related to methionine and one-carbon metabolism, has been searched recently. Glycine N-methyltransferase (GNMT) is the main component that affects the sarcosine syntheses (17-19). GNMT syntheses is controlled with the same-named gene. It has been recently identified that this gene is on the sixth chromosome's short arm's 12th position (19). The increase of GNMT production causes glycine to transform sarcosine and increase its excretion in urine. Stabler et al. (20) have proven that the increased GNMT increases homocysteine and sarcosine formation with increasing s-adenosyl methionine usage. This fact caused sarcosine to be thought as a bioreagent in the non-invasive cancer field.

In 2009, Sreekumar et al. (21), examined metabolomic characters of PC and suggested that urine sarcosine levels could be used as reagent for prediction of PC progression. After this study, number of studies about this subject increased and many studies with different methods were performed. Some of those studies supported sarcosine as a reagent in cancer determination; however, some of them didn't.

Table 3. Assessment of colorimetric sarcosine measurements and fluorometric sarcosine measurements according to pathology result

	Pathology		[a]p
	Benign (n=60)	Malignant (n=24)	
	Median (min-max)	Median (min-max)	
Colorimetric sarcosine	0.41 (0-1.2)	0.44 (0.01-0.9)	0.365
Fluorometric sarcosine	0.30 (0-1.3)	0.40 (0-0.9)	0.513

[a]Mann-Whitney U test, min: minimum, max: maximum

Urine sarcosine levels were evaluated with colorimetric and fluorometric methods in our study, which aimed to determine the efficiency of sarcosine in predicting PC, especially in patients with low-level PSA. There was no statistically significant difference detected between the benign and malignant patients in terms of urine sarcosine levels (p>0.05). Our finding matches Jentzmik et al.'s (6) and Struys et al.'s (7) studies in literature, however, it doesn't match with Bianchi et al.'s (22) and Cernei et al.'s (5) studies.

In Jentzmik et al.'s (6) study in 2010, the urine sarcosine levels were evaluated with gas chromatography and spectrometry in 106 patients with PC and 33 controls without cancer. It was seen that sarcosine/creatinine ratio was 13% less in patients with PC. As a result, it has no additional extender to PSA in benign-malignant differentiation and it's more inadequate than fPSA (6).

Struys et al. (7) reported that there was no significant difference between patients with increased serum PSA level, patients with metastatic PC and the control group (patients were chosen from patients whose vitamin B12 levels were examined). They even identified that serum sarcosine levels were not helpful with serum PSA level increase; therefore, it wasn't helpful in prediction of cancer progression.

In 2011, Bianchi et al. (22) evaluated urine sarcosine levels of a total of 56 participants consisting of healthy controls, patients with benign prostate hypertrophy (BPH) and patients with prostate gland localized cancer with fully automated solid-phase microextraction-fast gas chromatography and mass spectrometry. They found that the sarcosine/creatinine ratio in stated participants were 103, 137, and 267 µg/g, respectively. The highest sensitivity was 79%, and specificity was 87% with cut-off sarcosine value of 179 µg sarcosine (g creatinine)$^{-1}$ and in case of usage of this cut-off value, sarcosine had an important relationship with the cancer presence (p<0.0001). The correlation between patients with clinical localized cancer and patients with no evidence of tumour was presented with receiver operating characteristic analysis (22).

Urine sarcosine values of patients with PC -were evaluated with ion-exchange chromatography developed by Cernei et al. (5), and it was seen that those patients had significantly higher sarcosine levels than treated patients. It was shown that urine sarcosine levels of healthy people could be ignored. In our study, which had a starting point accordingly, it was identified that patients who were reported as having BPH and healthy people had sarcosine in their urines. There was no statistically significant difference found in malignant patients, and our study didn't reveal the fact that sarcosine levels in healthy people could be ignored.

Koutros et al. (8), examined sarcosine levels of 1,122 patients with

Table 4. Assessment of colorimetric sarcosine measurements and fluorometric sarcosine measurements in the cases according to pathology result

	Benign (n=60)		Malignant (n=24)	
	r	p	r	p
Pre-biopsy PSA - colorimetric sarcosine	0.071	0.591	-0.013	0.953
Pre-biopsy PSA - fluorometric sarcosine	0.018	0.894	0.149	0.487
PSA on the day of the biopsy - colorimetric sarcosine	0.118	0.369	-0.176	0.410
PSA on the day of the biopsy - fluorometric sarcosine	0.127	0.335	-0.025	0.906
Percentage change in PSA (%) - colorimetric sarcosine	0.211	0.189	-0.415	0.044*
Percentage change in PSA (%) - fluorometric sarcosine	0.318	0.013*	-0.418	0.042*

r=Spearman's correlation coefficient, *p<0.05, PSA: prostate specific antigen

Table 5. Assessment of PSA, sarcosine, prostate volume and prostatitis parameters in the cases with malignant pathology result according to content Gleason score

		Gleason score 6	Gleason score 7	
		Median (min-max)	Median (min-max)	p
Pre-biopsy PSA		6.12 (3.35-9.50)	5.87 (2.57-8.68)	[a]0.696
PSA on the day of the biopsy		6.16 (3.21-10.51)	5.65 (3.67-10.30)	[a]0.804
Percentage change in PSA (%)		0.13 (-29.51/50.57)	13.51 (-3.75/42.8)	[a]0.166
Colorimetric sarcosine		0.42 (0.01-0.85)	0.57 (0.26-0.69)	[a]0.374
Fluorometric sarcosine		0.30 (0-0.9)	0.40 (0.2-0.7)	[a]0.389
Prostate volume		32.40 (18-140)	40.0 (20-75)	[a]0.749
		n (%)	n (%)	
Prostatitis	Present	8 (42.1%)	0 (0.0%)	[b]0.130
	Absent	11 (57.9%)	5 (100.0%)	

[a]Mann-Whitney U test, [b]Fisher Exact test, min: minimum, max: maximum, PSA: prostate specific antigen

J Acad Res Med 2022;12(2):42-8

PC (813 non-aggressive and 309 aggressive) and 1,112 controls with liquid chromatography-mass spectrometry. They found that as the sarcosine level increased, PC risk increased (p=0.03). As a result, Koutros et al. (8) reported that high serum sarcosine levels accompanied increased PC risk and that sarcosine could be used as bioreagent.

Our findings support most of the studies in literature. For example, Koutros et al. (8) classified PC according to tumor aggressiveness in 4 groups (Q1-Q4) and evaluated the relationship between sarcosine levels and the aggressiveness of the disease. They reported strong relationship in non-aggressive patients [for Q4-Q1 odds ratio =1.44, 95% confidence interval (CI): 1.11, 1.88; P-trend 0.006]. However they didn't report a significant relationship in the aggressive patients (for Q4-Q1 odds ratio =1.03, 95% CI: 0.73, 1.47; P-trend 0.89).

Cao et al. (12) identified that urine sarcosine and sarcosine/creatinine ratio were incompatible with Gleason score and T phase. Similarly, Jentzmik et al. (6) found that sarcosine levels weren't related to tumor phase or Gleason score (<7 vs. ≥7) (19). In our study, there was no statistically significant difference between colorimetric and fluorometric sarcosine measurements of the malignant patients according to Gleason scores as well (p>0.05).

Struys et al. (7), reported that sarcosine levels weren't correlated with tumor progression. Wu et al. (4) showed that sarcosine/creatinine ratio wasn't sufficient for cancer diagnosis and wasn't determinant for histological degree and identifying the tumour behavior. According to those studies and our study, sarcosine levels and tumor aggressiveness are not related and disease aggression cannot be evaluated with sarcosine levels.

Apart from all these data, our study found statistically significant negative correlation in malignant group and positive correlation in benign group with percentage change in PSA values and fluorometric sarcosine measurements.

Study Limitations

The main limitation was the low amount of sample size. However, the study was designed to be prospective, all groups' features were analyzed without a control group. Sarcosine kit was obtained from an abroad country and only 90 kit contents could be received. Sample storage had to be in -40 °C and the limiting storage time was maximum 3 months. Despite all these challenging difficulties and lack of technical issues, we believe that we have designed a good study to make an addition to the present literature.

CONCLUSION

In our study, which we used not only fluorometric technic but also colorimetric technic, sarcosine levels were found inadequate in predicting PC, differentiating benign-malignant patients, and predicting the aggressiveness of the disease. However, the correlation between percentage change in PSA values and fluorometric sarcosine measurements might be used in grey zone PSA patients. Combining this correlation with newly popular Multiparametric Prostate MR results may lead us avoid unnecessary biopsies in especially patients with low level PSA and PI-RADS 2-3.

Acknowledgement

The authors would like to thank Yedikule Hıfzıssıhha Institute for the permission to use their laboratories.

Ethics Committee Approval: Study was performed between 15.12.2013-15.03.2014 after the Taksim Training and Research Hospital Clinical Research Ethics Committee approval (decision no: 29, date: 04.12.2013).

Informed Consent: All patients gave their informed consent for participation.

Peer-review: Externally and internally peer-reviewed.

Author Contributions: Surgical and Medical Practices - O.F., N.N.; Concept - O.F., N.N., B.N.; Design - O.F., M.B.C.B., M.A.; Data Collection and/or Processing - O.F., N.N., A.E., A.K., C.T.G.; Analysis and/or Interpretation - O.F., A.K., B.N.; Literature Search - O.F., A.E., A.K., C.T.G.; Writing - O.F.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, et al. EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014; 65: 124-37.
2. Taha DE, Aboumarzouk OM, Koraiem IO, Shokeir AA. Antibiotic therapy in patients with high prostate-specific antigen: Is it worth considering? A systematic review. Arab J Urol 2019; 18: 1-8.
3. Lucarelli G, Fanelli M, Larocca AM, Germinario CA, Rutigliano M, Vavallo A, et al. Serum sarcosine increases the accuracy of prostate cancer detection in patients with total serum PSA less than 4.0 ng/ml. Prostate 2012; 72: 1611-21.
4. Wu H, Liu T, Ma C, Xue R, Deng C, Zeng H, et al. GC/MS-based metabolomic approach to validate the role of urinary sarcosine and target biomarkers for human prostate cancer by microwave-assisted derivatization. Anal Bioanal Chem 2011; 401: 635-46.
5. Cernei N, Zitka O, Ryvolova M, Adam V, Masarik M, Hubalek J, et al. Spectrometric and electrochemicalanalysis of sarcosineas a potential prostate carcinoma marker. International Journal of Electrochemical Science 2012; 7: 4286-301.
6. Jentzmik F, Stephan C, Miller K, Schrader M, Erbersdobler A, Kristiansen G, et al. Sarcosine in urine after digital rectal examination fails as a marker in prostate cancer detection and identification of aggressive tumours. Eur Urol 2010; 58: 12-8; discussion 20-1.
7. Struys EA, Heijboer AC, van Moorselaar J, Jakobs C, Blankenstein MA. Serum sarcosine is not a marker for prostate cancer. Ann Clin Biochem 2010; 47: 282.
8. Koutros S, Meyer TE, Fox SD, Issaq HJ, Veenstra TD, Huang WY, et al. Prospective evaluation of serum sarcosine and risk of prostate cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Carcinogenesis 2013; 34: 2281-5.
9. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.
10. Patel S, Issa MM, El-Galley R. Evaluation of novel formula of PSA, age, prostate volume, and race in predicting positive prostate biopsy findings. Urology 2013; 81: 602-6.
11. Schostak M, Schwall GP, Poznanović S, Groebe K, Müller M, Messinger D, et al. Annexin A3 in urine: a highly specific noninvasive marker for prostate cancer early detection. J Urol 2009; 181: 343-53.
12. Cao DL, Ye DW, Zhang HL, Zhu Y, Wang YX, Yao XD. A multiplex model of combining gene-based, protein-based, and metabolite-based with positive and negative markers in urine for the early diagnosis of prostate cancer. Prostate 2011; 71: 700-10.
13. Dabir PD, Ottosen P, Høyer S, Hamilton-Dutoit S. Comparative analysis of three- and two-antibody cocktails to AMACR and basal cell markers

for the immunohistochemical diagnosis of prostate carcinoma. Diagn Pathol 2012; 7: 81.

14. Fernández-Serra A, Rubio-Briones J, García-Casado Z, Solsona E, López-Guerrero JA. Cáncer de próstata: la revolución de los genes de fusión [Prostate cancer: the revolution of the fusion genes]. Actas Urol Esp 2011; 35: 420-8.
15. Vesprini D, Liu S, Nam R. Predicting high risk disease using serum and DNA biomarkers. Curr Opin Urol 2013; 23: 252-60.
16. Pin E, Fredolini C, Petricoin EF 3rd. The role of proteomics in prostate cancer research: biomarker discovery and validation. Clin Biochem 2013; 46: 524-38.
17. Yen CH, Lin YT, Chen HL, Chen SY, Chen YM. The multi-functional roles of GNMT in toxicology and cancer. Toxicol Appl Pharmacol 2013; 266: 67-75.
18. Velichkova P, Himo F. Methyl transfer in glycine N-methyltransferase. A theoretical study. J Phys Chem B 2005; 109: 8216-9.
19. Soriano A, Castillo R, Christov C, Andrés J, Moliner V, Tuñón I. Catalysis in glycine N-methyltransferase: testing the electrostatic stabilization and compression hypothesis. Biochemistry 2006; 45: 14917-25.
20. Stabler S, Koyama T, Zhao Z, Martinez-Ferrer M, Allen RH, Luka Z, et al. Serum methionine metabolites are risk factors for metastatic prostate cancer progression. PLoS One 2011; 6: e22486.
21. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 2009; 457: 910-4.
22. Bianchi F, Dugheri S, Musci M, Bonacchi A, Salvadori E, Arcangeli G, et al. Fully automated solid-phase microextraction-fast gas chromatography-mass spectrometry method using a new ionic liquid column for high-throughput analysis of sarcosine and N-ethylglycine in human urine and urinary sediments. Anal Chim Acta 2011; 707: 197-203.