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VITAMIN D AND ANDROGENS IN PROSTATE DISEASE PATIENTS

***[1]Bistra Tzaneva Galunska, [1]Bogdan Roussev Hadjiev, [2]Daniela Ivanova Gerova, [3]Alexander Ivanov Hinev, [4]Natalya Vasilevna Usheva and [5]Dobrin Avramov Svinarov**

[1]Department of Biochemistry, Molecular Medicine and Nutrigenomics, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna Medical University – Varna, 55 Marin Drinov street, 9002 Varna, Bulgaria.
[2]Department of General Medicine and Clinical Laboratory, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna
[3]Department of Surgery, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.
[4]Department of Social Medicine and Healthcare Organization, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.
[5]Department of Clinical Laboratory and Clinical Pharmacology, Medical University Sofia, Bulgaria; Alexandrovska Hospital – Sofia, 1 St. Georgi Sofiyski street, 1431 Sofia, Bulgaria.

***Corresponding Author: Bistra Tzaneva Galunska**
Department of Biochemistry, Molecular Medicine and Nutrigenomics, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna Medical University – Varna, 55 Marin Drinov street, 9002 Varna, Bulgaria.

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ABSTRACT

Aim: To study the relationship between vitamin-D status, androgens and clinical determinants for benign and malignant prostate diseases. **Materials and methods:** This prospective single-centred study includes 54 benign prostatic hyperplasia patients age-matched with 88 prostate cancer patients, selected by serum prostate specific antigen levels, digital rectal examination, and systemic transrectal tru-cut prostate biopsies. The cancer patients were stratified by the risk for biochemical recurrence after definite local treatment and by tumour's Gleason score. Total and free testosterone, dehydroepiandrosterone sulphate, androstenedione, sex-hormone binding globulin, 25-hydroxyvitamin-D, and vitamin-D binding protein were measured. **Results:** Vitamin-D deficiency was more frequent in cancer patients vs noncancerous (69.4% vs 43.4%). Negative correlation ($r=-0.255$, $p<0.05$) and decrease ($p=0.07$) with the tumour grade was found for 25-hydroxyvitamin-D. Similar relationships were detected for free and total testosterone ($p=0.08$) and free androgen index ($r=-0.262$, $p<0.05$). Risk stratification revealed a decrease in 25-hydroxyvitamin-D ($p<0.05$) and vitamin-D binding protein (ns), while sex-hormone-binding protein ($p<0.01$) and prostate specific antigen/total testosterone ratio ($p<0.05$) were increased. Negative correlation between 25-hydroxyvitamin-D ($r=-0.21$, $p=0.05$) and free androgen index ($r=-0.23$, $p<0.05$) with the risk was detected. ROC curve analysis revealed that among tested steroids 25-hydroxyvitamin-D exhibited highest area under curve (AUC 0.69, $p<0.001$). Multifactorial regression analysis indicated prostate specific antigen ($\beta=0.49$, $p<0.0001$) and 25-hydroxyvitamin-D ($\beta=-0.24$, $p<0.04$) as statistically significant variables related to tumour grade. **Conclusion:** It might be hypothesized that prostate cancer is associated with disturbances in steroid metabolism. Development of steroid constellations, focused on vitamin-D, could be helpful for the assessment of disease progression and aggressiveness.

KEYWORDS: vitamin D, androgens, prostate disease.

INTRODUCTION

Prostate diseases are leading causes for morbidity and mortality among aging men. Benign prostate hyperplasia (BPH) affects about half of all men between 51 and 60 years and prostate cancer (PCa) is the second leading cause for cancer deaths in men.[1,2] Considerable interest has been recently demonstrated in re-evaluation the role of different steroid hormones on the onset, progression and outcome of prostate diseases. The belief that androgens can cause prostate growth and accelerate PCa has been replaced nowadays by the hypothesis that only low concentrations of testosterone may stimulate PCa growth and aggressiveness.[3] The antiproliferative effect of the steroidal hormone calcitriol promotes intensive research in the last decade seeking relationships between prostate growth, tumour aggressiveness and vitamin-D deficiency.[4]

The aim of the present study was to seek relationships between vitamin D status, androgen levels and various clinical determinants associated with the severity and progression of benign and malignant prostate diseases.

MATERIALS AND METHODS

Patients

The present single-centred study enrolled 142 men, (52-

85 years), with clinical suspicion for PCa, evoked by elevated serum prostate specific antigen (PSA) and/or abnormal digital rectal examination, who visited the Urology Clinic at the University Hospital-Varna within January-November, 2015. Patients with acute prostatitis, systemic infection, cardiorespiratory failure, absolute contraindications for surgical treatment, and vitamin-D supplementation were excluded from the study. All patients were subjected to systemic transrectal ultrasound-guided tru-cut prostate biopsies (10 cores at least). The biopsy specimens were processed by standard histological and immunohistochemical examinations. All malignant tumours were staged by the TNM classification (v. 2009) and graded by the Gleason grading system. Information, regarding patients' age and family history of PCa/BPH was collected at admission.

Study design

The biopsy results stratified the patients into two groups: benign (BPH) and malignant (PCa). The PCa group was subdivided by the risk for biochemical recurrence (BCR) and by the tumour's Gleason score. Three risk groups (RG) were defined according to the current EAU guidelines criteria: low risk (RG1, n=8, 9%); intermediate risk (RG2, n=37, 42%); high risk group (RG3, n=4, 49%).[5] Tumours with Gleason score <7 were defined as low grade (Gl1, n=22, 25%), those with Gleason score ≥7 – high grade (Gl2, n=66, 75%).

Laboratory examinations

The tested laboratory parameters were measured only once at patient admission. Serum samples were collected and stored at -80^{o}C until the time of analysis.

Total testosterone (TT), free testosterone (FT), dehydroepiandrosterone sulphate (DHEAS), androstenedione (A), and sex hormone binding globulin (SHBG), and vitamin-D binding protein (VDBP) were assayed by commercial ELISA kits (IVD Demeditec Diagnostics GmbH, Lise-Meitner-Str. 24145 Kiel, Germany). All ELISA readings were done on Synergy2 ELISA-reader (BioTek US). Free androgen index (FAI) was calculated as ratio TT/SHBG multiplied by 100.

Vitamin-D status was evaluated by the serum levels of 25-hydroxyvitamin-D (25OHD) measured by liquid chromatography with mass-selective detection (LC-MS/MS).[6]

PSA serum levels were measured by chemiluminescent immunometric assay using IMMULITE 2000 automated system.

Statistics

Continuous variables were analysed by one-way ANOVA and presented as mean ± SD. Categorical variables were presented as frequencies. Non-parametric Spearman correlation analysis was performed to evaluate the associations between tested parameters and clinical characteristics using GraphPad Prism, La Jolla, USA. SPSS for Windows, v.19. was used to construct linear regression model, equating the relationships between biochemical characteristics (TT/DHEAs, FT, PSA, 25OHD, A, PSA/TT, BMI, FAI, DHEAs, TT, age, season) and tumour grade (Gleason's score). The level of significance was set at $p<0.05$.

The study was approved by the local Ethics Committee, following the guidelines of the Declaration of Helsinki. Written informed consent was obtained by each patient, participating in the study.

RESULTS

The characteristics of the studied patients are given in Table 1.

Table 1: Characteristics of the studied patients.

Parameter	BPH mean±SD (n)	PCa mean±SD (n)	p
Age (years)	67.91±7.21 (54)	66.76±6.25 (88)	ns
BMI (kg/m^2)	26.38±3.18 (54)	27.53±4.13 (88)	ns
25OHD (nmol/L)	56.63±21.71 (52)	42.85±18.88 (85)	p<0.0001
VDBP	304.00±82.86 (40)	303.6±79.90 (64)	ns
TT (nmol/L)	12.80±4.57 (54)	10.51±4.25 (87)	p<0.01
FT (pmol/L)	32.08±10.03 (54)	26.98±10.48 (87)	p<0.01
Androstenedione (nmol/L)	5.92±2.70 (54)	5.52±2.55 (88)	ns
DHEAS (µmol/L)	2.31±0.93 (54)	2.74±1.21 (88)	p<0.05
SHBG (nmol/L)	72.32±32.85 (39)	73.24±44.83 (74)	ns
PSA (ng/ml)	5.38±4.39 (52)	30.99±42.20 (88)	p<0.0001
TT/DHEAS	6.62±3.91 (54)	6.07±8.92 (88)	ns
PSA/TT	1.76±2.17 (52)	15.21±28.22 (87)	p<0.0001
FAI=TT/SHBG x 100	20.37±14.94 (39)	19.1±12.43 (73)	ns

Abbreviations: BPH – benign prostate hyperplasia, PCa – prostate cancer, BMI – body mass index, 25OHD – 25-hydroxyvitamin D, VDBP – vitamin D binding protein, TT – total testosterone, FT – free testosterone, DHEAS - dehydroepiandrosterone, SHBG – sex-hormone binding globulin, PSA – prostate specific antigen, FAI – free androgen index; ns – non-significant.

Significant differences in PCa versus BPH group were found for 25OHD, TT, FT, DHEAS, PSA, and for PSA/TT. Vitamin-D deficiency (<50nmol/L) was more frequent in PCa than BPH patients – 69.4% vs 43.4%. Optimal vitamin-D status (25OHD>75nmol/L) was established in 16.9% of BPH and in only 4.7% of PCa patients. Among all measured androgens TT and FT revealed a significant decrease in the PCa group compared to BPH, while DHEAS was increased in PCa patients. The percentage of PCa patients with TT below accepted threshold for hypogonadism of 12nmol/L was higher than the percent of BPH cases (68.2% vs 44.4%, respectively). The FT values of PCa group were lower than BPH group values by 17%. No differences were detected in SHBG between these two groups of patients.

BPH patients

The stratification of BPH patients by the cut-off value for PSA (4ng/mL) revealed no changes in all tested parameters. When the patients were divided by the cut-off value for 25OHD (50nmol/L), no changes were detected for PSA, all tested androgens, free androgen index, and PSA/TT ratio.

No causal relationships were found between studied parameters and PSA as approved laboratory marker for prostate diseases. Having in mind the widely discussed antiproliferative effects of 25OHD, we studied its relationship with the tested steroids. No correlation was detected between the tested steroids and 25OHD.

PCa patients

As initial PSA<20ng/mL is accepted as a cut-off for bone metastases [7], we stratified the PCa patients into two groups by this cut-off value. Significant difference was found only for PSA/TT between these two groups (3.58±2.16 for PSA<20ng/mL vs 34.23±39.14 PSA≥20ng/mL). A weak negative correlation between PSA and FT (Spearman r = -0.28, p=0.07) was found for the entire PCa group.

The stratification by Gleason score ≥7 and below 7 demonstrated a significant decrease in 25OHD, TT, FT and increase in PSA and PSA/TT with the tumour grade (Table 2).

Table 2: Changes in the tested parameters according to the Gleason score.

Parameter	Gleason score <7 mean±SD (n)	Gleason score ≥7 mean±SD (n)	p
BMI (kg/m^2)	28.03±3.68 (21)	27.36±4.28 (64)	ns
25OHD (nmol/L)	46.23 ± 21.91 (21)	41.74 ± 17.83 (64)	p=0.07
VDBP (mg/L)	312.30 ± 56.94 (17)	298.60 ± 86.94 (47)	ns
TT (nmol/L)	11.29 ± 3.68 (21)	10.27 ± 4.42 (66)	p=0.08
FT (pmol/L)	28.67 ± 10.65 (21)	26.44 ± 10.45 (66)	ns
Androstenedione (nmol/L)	4.68 ± 2.02 (22)	5.80 ± 2.66 (66)	ns
DHEAS (µmol/L)	2.49 ± 1.04 (22)	2.82 ± 1.26 (66)	ns
SHBG (nmol/L)	74.45 ± 54.33 (18)	72.85 ± 41.88 (56)	ns
PSA (ng/ml)	15.15 ± 14.17 (22)	36.27 ± 41.83 (66)	p<0.0001
TT/DHEAS	6.54 ± 8.01 (21)	6.01 ± 9.28 (66)	ns
PSA/TT	5.04± 4.81 (21)	18.44 ± 31.66 (66)	p<0.01
FAI=TT/SHBG x 100	20.92 ± 9.29 (16)	18.58 ± 13.21 (57)	ns

Abbreviations: PCa – prostate cancer, BMI – body mass index, 25OHD – 25-hydroxyvitamin D, VDBP – vitamin D binding protein, TT – total testosterone, FT – free testosterone, DHEAS - dehydroepiandrosterone, SHBG – sex-hormone binding globulin, PSA – prostate specific antigen, FAI – free androgen index; ns – non-significant.

The relationships between the Gleason score and the tested parameters revealed a significant negative correlation for 25OHD (p<0.05) and FAI (p<0.05), a positive relationship for PSA (p<0.0001) and PSA/TT (p<0.0001), and a negative borderline link for TT (p=0.065) (**Fig. 1A, B, C**).

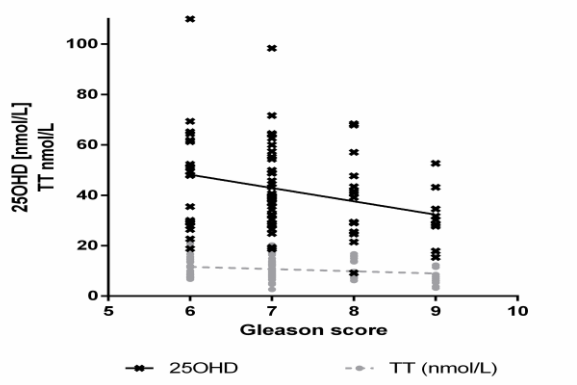

Figure 1A: Correlation between Gleason score, 25OHD and TT in the studied PCa patients.

Abbreviations: PCa – prostate cancer, 25OHD – 25-hydroxyvitamin D, TT – total testosterone. Non-parametric Spearman correlation analysis was used. Spearman r = - 0.26, p<0.05 for 25OHD; Spearman r = - 0.20, p=0.06 for TT.

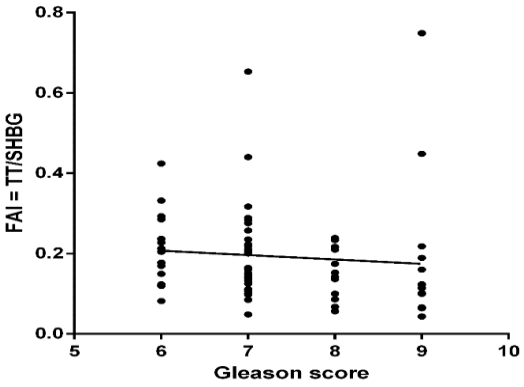

Figure 1B: Correlation between Gleason score and FAI in the studied PCa patients.

Abbreviations: PCa – prostate cancer, FAI – free androgen index. Non-parametric Spearman correlation analysis was used. Spearman r = - 0.26, p<0.05 for FAI.

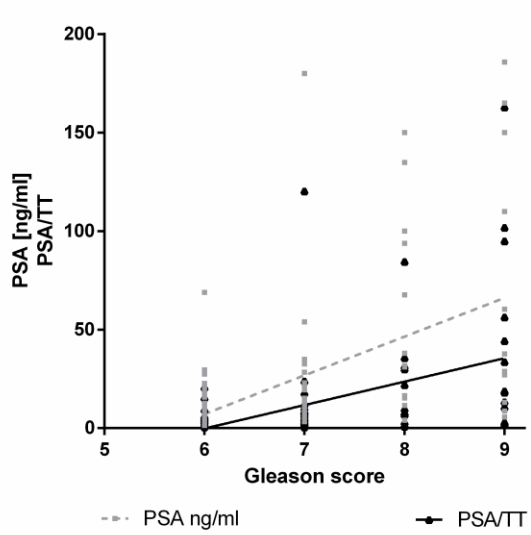

Figure 1C: Correlation between Gleason score, PSA and PSA/TT in the studied PCa patients.

Abbreviations: PCa – prostate cancer, TT – total testosterone, PSA – prostate specific antigen. Non-parametric Spearman correlation analysis was used. Spearman r = 0.40, p<0.0001 for PSA; Spearman r = 0.41, p<0.0001 for PSA/TT.

When the PCa patients were distributed by BCR risk, a decrease in 25OHD, VDBP, and TT levels was detected, while SHBG and PSA/TT ratio were increased with the risk (Table 3).

Table 3: Changes in the tested parameters according to the risk of biochemical recurrence.

Parameter	RG1(low risk) mean±SD (n)	RG2 (intermediate risk) mean±SD (n)	RG3 (high risk) mean±SD (n)	p
BMI (kg/m^2)	27.7±4.39 (8)	27.39±3.71 (37)	27.62±4.51 (43)	ns
25OHD (nmol/L)	40.69±19.32 (7)	47.87±18.57 (37)	38.69±18.45 (41)	RG2 vs RG3 p<0.05
VDBP (mg/L)	325.1±64.75 (5)	288.9±64.77 (32)	317.0±96.34 (27)	RG1 vs RG2 p<0.001 RG2 vs RG3 p<0.0001
TT (nmol/L)	12.44±4.98 (8)	9.68±3.55 (36)	10.85±4.58 (43)	RG1 vs RG2 p=0.07
FT (pmol/L)	29.63±12.3 (8)	25.47±8.78 (36)	27.75±11.47 (43)	ns
Androstenedione (nmol/L)	5.11±1.99 (8)	5.27±2.36 (37)	5.81±2.81 (43)	ns
DHEAS (µmol/L)	2.33±0.94 (8)	2.72±1.24 (37)	2.83±1.23 (43)	ns
SHBG (nmol/L)	64.7±25.53 (6)	61.36±36.70 (31)	79.68±41.91 (36)	RG2 vs RG3 p<0.01
PSA (ng/ml)	12.02±8.34 (8)	18.78±24.17 (37)	45.03±52.69 (43)	RG1 vs RG3 p<0.001 RG2 vs RG3 p<0.0001
TT/DHEAS	6.53±3.96 (8)	6.77±11.01 (36)	5.54±7.72 (43)	ns
PSA/TT	3.25±1.75 (8)	6,634±7,037(35)	22.83±36.5 (43)	RG1 vs RG3 p<0.05 RG2 vs RG3 p<0.01
FAI=TT/SHBG x 100	20.09±6.98 (6)	20.99±12.72 (31)	17.30±12.87 (36)	ns

Abbreviations: PCa – prostate cancer, BMI – body mass index, 25OHD – 25-hydroxyvitamin D, VDBP – vitamin D binding protein, TT – total testosterone, FT – free testosterone, DHEAS - dehydroepiandrosterone, SHBG – sex-hormone binding globulin, PSA – prostate specific antigen, FAI – free androgen index; ns – non-significant.

A negative correlation between FAI (p<0.05), as well as between 25OHD (p=0.05) and the degree of BCR risk was found (Figure 2 A, B).

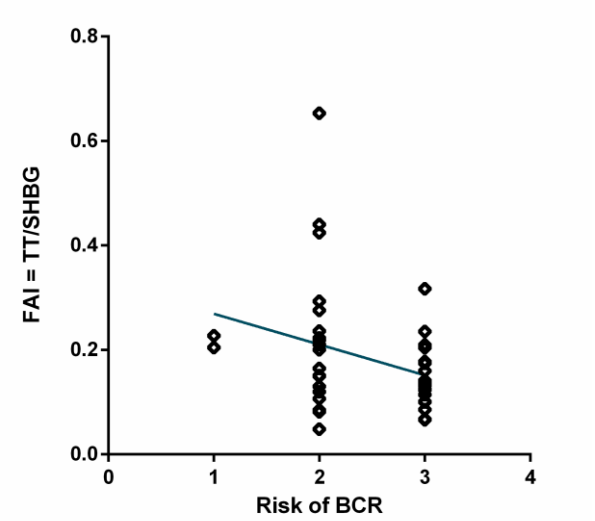

Figure 2A: Correlation between the risk for biochemical recurrence and FAI in the studied PCa patients.

Abbreviations: BCR – biochemical recurrence, PCa – prostate cancer, FAI – free androgen index. Non-parametric Spearman correlation analysis was used. Spearman r = - 0.23, p<0.05.

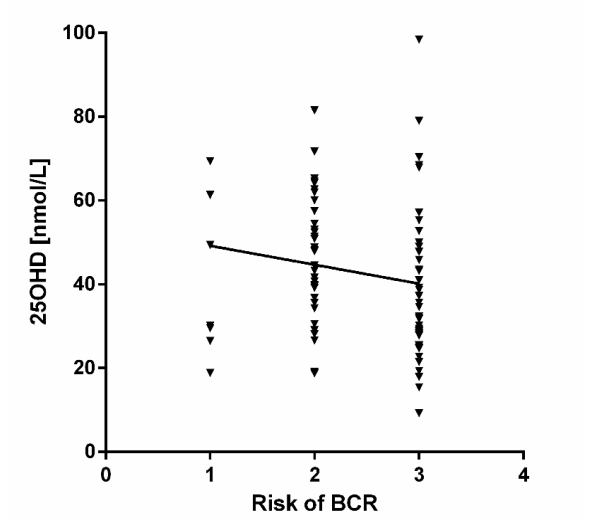

Figure 2B: **Correlation between the risk for biochemical recurrence and 25OHD in the studied PCa patients.**

Abbreviations: BCR – biochemical recurrence, PCa – prostate cancer, 25OHD – 25-hydroxyvitamin D. Non-parametric Spearman correlation analysis was used. Spearman r = - 0.21, p=0.05.

The stratification by the cut-off value 50nmol/L for 25OHD showed significantly higher PSA levels for the vitamin D deficient group vs vitamin-D sufficient one (33.78±46.82ng/mL vs 24.34±27.99ng/mL, p<0.05). On the contrary, VDBP was decreased in the vitamin D deficient PCa patients (266.9±61.09mg/L vs 335.3±80.64mg/L, p<0.0001). There were no significant changes in other tested steroids between PCa groups stratified by the vitamin D status.

ROC curve analysis revealed that among all tested steroids 25OHD exhibited highest area under curve (AUC=0.69, p<0.001) followed by TT/DHEAs (AUC=0.67, p<0.001) TT and FT (AUC=0.65, p<0.05), and DHEAs (AUC=0.61, p<0.05).

Linear regression analysis indicated two statistically significant variables, PSA (β=0.49, p<0.0001) and 25OHD (β=-0.24, p<0.04) related to tumour grade.

DISCUSSION

There is no consensus on serum androgen levels in men suffering from different prostate diseases. Regarding the "androgen hypothesis", high testosterone levels promote the PCa development and progression, while low testosterone could have a protective role. Recently the androgen hypothesis was challenged by the "saturation model", stating that prostate tissue is more sensitive to changes in androgens at low concentrations and becomes indifferent to changes at high concentrations.[3] It is considered that the most potent natural androgen dihydrotestosterone (DHT) could play an imminent role in adult prostate and is a prime suspect in uncontrolled proliferation and prostate enlargement.[8]

In our study the PCa patients' data were evaluated, juxtaposing them to the data of the BPH patients. The levels of TT, FT were decreased in PCa patients in comparison to the BPH group. There are conflicting data regarding the serum levels of androgens in BPH and PCa. Similarly to our results, Mearini *et al*. established significantly lower testosterone levels in patients with PCa compared to those with BPH.[9] On the contrary, Usoro *et al*. detected significantly higher testosterone levels in 62 PCa patients compared to 116 BPH patients.[10] It is considered that serum FT correlates with the prostatic androgen environment better than TT.[11] In our patients, FT levels were significantly lower in PCa than in BPH. Travis *et al.* also found a decreased FT in PCa patients vs. control subjects.[12] The concentration of TT and FT is regulated by numerous factors. Some of them are DHEA and androstenedione as precursors for testosterone synthesis. In addition, the concentration of FT is tightly dependent on the availability of SHBG.[13] The affinity of androgens for binding SHBG is higher for DHT and lower for testosterone. It is believed that SHBG modulates the ratio between free, SHBG-bound and albumin-bound fractions, regulates steroid bioavailability and acts not only as a controlling factor for sex hormone balance, but also as an amplifier of sex steroid effects in general. Increased SHBG levels might be related to reduced bioactivity of androgens. Having in mind the regulatory effects of SHBG on sex steroid balance, it might be hypothesized that SHBG is related to the development of hormone-dependent tumours, including PCa.[14] In our study, we found slight but non-significant increase in SHBG in PCa patients vs BPH group. The

same tendency was confirmed in other studies indicating significantly higher SHBG levels in PCa vs. noncancerous patients.[15,16] On the contrary, others did not found differences in SHBG levels between PCa patients and controls.[17]

The androgen disbalance detected in our PCa patients (significantly high DHEAS, decreased TT and FT, and unchanged androstenedione) suggest that the activity of some enzymes involved in androgen metabolism in prostate gland might be altered. Several studies described three pathways for DHT generation – the active form of testosterone. It is considered that in prostate cancer cells the alternative pathways bypassing testosterone formation are more active than the classical pathway.[18]

Another steroid, intensively studied in relation to its antiproliferative effects, is the biologically active form of vitamin D_3.[19] Our study demonstrated a significant decrease in 25OHD values in PCa patients' vs BPH group. Moreover, the vitamin-D deficiency (25OHD<50nmol/L) prevailed in the PCa group. Similarly, Choo *et al*. established high rate of vitamin-D insufficiency among patients with non-metastatic PCa using a cut-off value of 75nmol/L.[20] On the contrary, others did not find any significant difference in vitamin-D levels between subjects with, and without PCa.[21]

25OHD deficiency in our PCa patients was related to significantly higher PSA. The same association was reported also by other authors.[22] It can be supposed that vitamin D deficient patients are more prone to prostate gland destruction estimated by higher levels of PSA. In our study, vitamin D deficiency was related to significantly higher VDBP levels probably due to compensatory mechanism in response to low 25OHD levels.

The relationships between different androgens and clinical parameters evaluating the severity and aggressiveness of PCa are studied also by other authors. However, the results are still contradictory. In our study, a weak borderline negative correlation between FT and PSA was found for the entire PCa group. FAI was used to determine abnormal androgen status in humans and is an indirect indicator of FT. Our results showed a week negative correlation between FAI and BCR risk and Gleason score. Similar negative relations between serum FT and disease aggressiveness, evaluated by the Gleason score and the BCR risk, were reported by Hoffman *et al*.[23] For other tested androgens, we did not find a causal link with the Gleason score, BCR risk and PSA.

Vitamin D signalling role in androgen metabolism is widely discussed in the literature. It is considered that calcitriol induces the expression of enzymes involved in the metabolism and inactivation of testosterone and androstanediol in prostate cells.[24] Other studies reported that calcitriol decreases the production of corticosterone, androstenedione, DHEA and DHEAS in human adrenocortical carcinoma cell line and upregulates the expression of 17ß-hydroxysteroid dehydrogenase type 2, 4, and 5 in human prostate cells.[25] Both DHEA and DHEAS are androgen precursors synthesized in the adrenal gland and their blood concentration is of significant importance for the androgen synthesis in the prostate cells.[13] It could be hypothesized that reduced 25OHD levels may result in disturbances in androgen metabolism in different prostate diseases.

Studying the relationships between 25OHD and tested androgens, we found a significant positive correlation only with the FT fraction for BPH (Spearman $r = 0.29$, $p<0.05$) and PCa (Spearman $r = 0.32$, $p<0.01$) patients.

Our results are in agreement with the data of Hofer et al, stating a direct stimulatory effect of calcitriol on testosterone production and up-regulation of steroidogenic enzymes expression in healthy human testicular cells.[26] Significant increase of TT, bioactive T, and FT was established in clinical settings after vitamin D supplementation (3332 IU/d).[27]

Beside the role of vitamin D in androgen regulation, it controls also prostate epithelial and cancer cells growth via its receptor (VDR) present in these cells. The results of some clinical studies support the hypothesis that vitamin D may contribute for slowing down the PCa progression.[28] Our results, revealing a significant negative correlations between 25OHD levels, Gleason score (Spearman $r = -0.26$, $p<0.05$), and BCR risk (Spearman $r = -0.21$, $p=0.05$) are in agreement with the data of Gilbert *et al*. stating that lower 25OHD concentrations are associated with a more aggressive PCa.[29]

The present study showed that 25OHD was the most sensitive parameter among tested steroids regarding its diagnostic reliability evaluated by ROC curve analysis. In terms of its relationship to the tumour aggressiveness, the linear regression demonstrates the essential role of vitamin D among the tested steroids.

It is important to underline that one of the limitations of our study is that the tested steroids were analysed only at patients' admission. For evaluation of the specific changes and causal links between the tested steroids and the clinical characteristics related to the malignant process, we used a group of age matched BPH patients. In this study, the androgen levels were measured by routine immunochemical methods and only 25OHD was assayed by LC-MS/MS, recommended as most relevant for steroid analysis. Not enough convincing interrelations between SHBG and vitamin D in our study were probably due to the smaller number of patients tested for SHBG. However, this study is the first one in our country, aiming to explore the relationship between different steroids, on the one hand, and the severity and progression of prostate diseases, on the other.

CONCLUSION

The present study supports the hypothesis that PCa is frequently associated with disturbances in steroid metabolism. The established causal links lead to the idea that not a single steroid is sufficient to assess the progression and aggressiveness of PCa. Future research on steroid constellations, focusing on vitamin D, could be helpful for the routine clinical practice.

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REFERENCES

1. Roehrborn CG. Benign prostatic hyperplasia: an overview. Rev Urol, 2005; 7(9): S3-S14.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer, 2013; 49(6): 1374-403.
3. Khera M, Crawford D, Morales A, Salonia A, Morgentaler A. A New Era of Testosterone and Prostate Cancer: From Physiology to Clinical Implications. European Urology, 2014; 65(1): 115-123.
4. Nyame YA, Murphy AB, Bowen DK, Jordan G, Batai K, Dixon M, Hollowell C, Kielb S, Meeks J, Gann P, Macias V, Kajdacsy-Balla A, Catalona W, Kittles R. Associations Between Serum Vitamin D and Adverse Pathology in Men Undergoing Radical Prostatectomy. Clin Oncol, 2016; 34(12): 1345-1349.
5. Heidenreich A, Bastian P, Bellmunt M, Bolla M, Joniau S, van der Kwast T, Mason M, Matveev V, Wiegel T, Zattoni F, Mottet N; European Association of Urology. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol, 2014; 65(2): 467-479.
6. Galunska B, Gerova D, Boncheva M, Svinarov D. HPLC method for measuring the circulating levels of 25-hydroxy vitamin D: Validation and comparison with ID LC/MS/MS and immunoassay. Integrative Food, Nutrition and Metabolism, 2014; 1(2): 1-6.
7. Heidenreich A. Identification of high-risk prostate cancer: role of prostate-specific antigen, PSA doubling time, and PSA velocity. Eur Urol, 2008; 54(5): 976-977.
8. Praaven R. Benign prostatic hyperplasia: updated review. International Research Journal of Pharmacy, 2013; 4(8): 45-51.
9. Mearini L, Costantini E, Zucchi A, Mearini E, Bini V, Cottini E, Porena M. Testosterone levels in benign prostatic hypertrophy and prostate cancer. Urol Int., 2008; 80(2): 134-140.
10. Usoro A, Obot A, Ekaidem I, Akaiso O, Udoh A, Akinloye O. Serum Testosterone, 17β-Estradiol and PSA Levels in Subjects with Prostate Disorders. Indian J Clin Biochem, 2015; 30(1): 59-65.
11. Regis L, Planas J, Carles J, Maldonado X, Comas I, Ferrer R, Morote J. Free Testosterone During Androgen Deprivation Therapy Predicts Castration-Resistant Progression Better Than Total Testosterone. Prostate, 2017; 77(1): 114-120.
12. Travis R, Key T, Allen N, Appleby PN, Roddam AW, Rinaldi S, Egevad L, Gann PH, Rohrmann S, Linseisen J, Pischon T, Boeing H, Johnsen NF, Tjønneland A, Overvad K, Kiemeney L, Bueno-de-Mesquita HB, Bingham S, Khaw KT, Tumino R, Sieri S, Vineis P, Palli D, Quirós JR, Ardanaz E, Chirlaque MD, Larrañaga N, Gonzalez C, Sanchez MJ, Trichopoulou A, Bikou C, Trichopoulos D, Stattin P, Jenab M, Ferrari P, Slimani N, Riboli E, Kaaks R. Serum androgens and prostate cancer among 643 cases and 643 controls in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer, 2007; 121(6): 1331-1338.
13. Chang K, Sharifi N. Prostate cancer - from steroid transformations to clinical translation. Nat Rev Urol, 2012; 9(12): 721-724.
14. Thaler M, Seifert-Klauss V, Luppa P. The biomarker sex hormone-binding globulin - from established applications to emerging trends in clinical medicine. Best Pract Res Clin Endocrinol Metab. 2015; 9(5): 749-760.
15. Garcia-Cruz E, Huguet J, Piqueras M, Perez Marquez M, Peri L, Izquierdo L, Franco A, AlvarezVijande R, Ribal MJ, Alcaraz A. Low testosterone bioavailability is related to prostate cancer diagnose in patients submitted to prostate biopsy. World J Urol., 2012; 30(3): 361-365.
16. De Nunzio C, Lombardo R, Albisinni S, Gacci M, Tubaro A.Serum levels of sex hormone binding globulin (SHBG) are not predictive of prostate cancer diagnosis and aggressiveness: results from an Italian biopsy cohort. Int Braz J Urol., 2013; 39(6): 793-799.
17. Sawada N, Iwasaki M, Inoue M, Sasazuki S, Yamaji T, Shimazu T, Tsugane S; Japan Public Health Center-based Prospective Study Group. Plasma testosterone and sex hormone-binding globulin concentrations and the risk of prostate cancer among Japanese men: a nested case-control study. Cancer Sci., 2010; 101(12): 2652-2657.
18. Mostaghel E. Steroid hormone synthetic pathways in prostate cancer. Transl Androl Urol, 2013; 2(3): 212-227.
19. Fleet J, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: a review of molecular mechanisms. Biochem J., 2012; 441(1): 61-76.
20. Choo C, Mamedov A, Chung M, Choo R, Kiss A, Danjoux C. Vitamin D insufficiency is common in patients with nonmetastatic prostate cancer. Nutr Res., 2011; 31(1): 21-6.

21. Yaturu S, Zdunek S, Youngberg B. Vitamin D Levels in Subjects with Prostate Cancer Compared to Age-Matched Controls. Prostate Cancer, 2012; 2012: 524206.
22. Grant W. Vitamin D status: ready for guiding prostate cancer diagnosis and treatment? Clin Cancer Res., 2014; 20(9): 2241-2243
23. Hoffman M, DeWolf W, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? J Urol., 2000; 63(3): 824-847.
24. Maguire O, Pollock C, Martin P, Owen A, Smyth T, Doherty D, Campbell MJ, McClean S, Thompson P. Regulation of CYP3A4 and CYP3A5 expression and modulation of "intracrine" metabolism of androgens in prostate cells by liganded vitamin D receptor. Mol. Cell. Endocrinol, 2012; 364(1-2): 54-64.
25. Lundquist J. Vitamin D as regulator of steroidogenic enzymes. F1000 Research, 2014; 3: 155: 1-10.
26. Hofer D, Münzker J, Schwetz V, Ulbing M, Hutz K, Stiegler P, Zigeuner R, Pieber TR, Müller H, Obermayer-Pietsch B. Testicular synthesis and vitamin D action. J Clin Endocrinol Metab, 2014; (10): 3766-73.
27. Pilz S, Frisch S, Koertke H, Kuhn J, Dreier J, Obermayer-Pietsch B, Wehr E, Zittermann A. Effect of vitamin D supplementation on testosterone levels in men. Horm Metab Res., 2011; 43(3): 223–225.
28. Lou Y, Qiao S, Talonpoika R, Syvälä H, Tuohimaa P. The role of Vitamin D3 metabolism in prostate cancer. J Steroid Biochem Mol Biol., 2004; 92(4): 317-325.
29. Gilbert R, Metcalfe C, Fraser W, Donovan J, Hamdy F, Neal D, Lane J, and Martin R. Associations of circulating 25-hydroxyvitamin D with prostate cancer diagnosis, stage and grade. Int J Cancer., 2012; 131(5): 1187-1196.