

IS VITAMIN D ASSOCIATED WITH TESTOSTERONE IN BENIGN PROSTATE HYPERPLASIA?

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ABSTRACT

INTRODUCTION: Benign prostate hyperplasia (BPH) affects about 50% of the male population between 51-60 years of age and almost 90% of the males aged 81-90. It is considered that BPH pathogenesis involves epithelial cells and stromal tissue proliferation inside prostate gland and testosterone is one of the promoting factors of prostate cell growth. Evidences about the antiproliferative effects of vitamin D and the widespread vitamin D deficiency and insufficiency among the Bulgarian population suggest a possible relation between vitamin D and testosterone in BPH patients.

AIM: The aim of this paper is to evaluate the vitamin D status and total testosterone (TT) levels in BPH patients and their associations with laboratory parameters such as prostate specific antigen (PSA) for prostate growth.

MATERIALS AND METHODS: A total of 37 male BPH patients (mean age 67.14 ± 7.77 years) were enrolled in the study. In all patients, BPH was histologically proven. PSA and TT levels were analyzed immunochemically. The circulating form of vitamin D, 25-hydroxyvitamin D (25OHD) was assayed by liquid chromatography with mass-spectrometry detection (LC-MS/MS). Other covariates (BMI, age,) were collected by interview at the time of hospitalization. Classical biochemical parameters were assayed by routine spectrophotometric tests. Descriptive statistics, variation and non-parametric correlation analysis were used. The level of significance was set at $p < 0.05$.

RESULTS: The mean level of 25OHD for BPH patients was close to the lower reference limit of 50nmol/L recommended by the US Endocrine Society Guideline. The majority of BPH patients (56.8%) display 25OHD levels above 50nmol/L, 43.2% of them were vitamin D deficient (25OHD < 50nmol/L), 8.1% – with severe vitamin D deficiency (25OHD < 25nmol/L), and only 6 patients (16.2%) had optimal 25OHD levels above the limit of 75 nmol/L. The mean serum TT levels of BPH patients were 10.74 ± 4.026 nmol/L, close to the lower limit of 10.4 nmol/L for normal TT, according to the recommendations of the Endocrine Society. A significant seasonal variations were found for 25OHD levels

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($p < 0.05$) between the cold and warm season. Similar seasonality was not established for TT. Two-thirds of BPH patients (62.9%) were with PSA values below the upper limit of the reference interval of 4 ng/ml. Higher 25OHD levels (59.21 ± 3.756 nmol/l, $p = 0.06$) were established for the group with PSA below the threshold of 4ng/ml. A moderate negative correlation (Spearman $r = -0.6707$, $p < 0.01$) was found only for the vitamin D deficient group. In case of vitamin D sufficiency, a weak positive trend was detected.

CONCLUSION: Our study indicated vitamin D insufficiency in BPH patients according to the criteria of the Endocrine Society. Strong negative correlation between 25OHD and TT levels was found for vitamin D deficient BPH patients. Higher 25OHD were associated with lower PSA values indicating a potential favorable effect of 25OHD on slackening of BPH.

Keywords: 25-hydroxyvitamin D, benigns prostate hiperplasia, testosterone

INTRODUCTION

Benign prostate hyperplasia (BPH) is a non-malignant excessive growth of prostate epithelial and stromal tissue. It leads to increased prostate volume and decreased flow of urine through the urethra (1). The prevalence of BPH is about 8% in men during their third decade, 50% in the fifth decade and 90% in the ninth decade of life (1,2,3).

There is accumulating evidence suggesting that vitamin D is involved in the pathogenesis of BPH. It has been shown that prostate epithelial cells express vitamin D receptor (VDR), mediating the biological actions of vitamin D and the enzyme 1-alpha hydroxylase, forming the active form of vitamin D, calcitriol (3). Calcitriol binds to VDR and stimulates the non-classical effects of vitamin D responsible for its anti-proliferative and pro-apoptotic actions on prostate cells. It has been demonstrated that calcitriol and its analogs inhibit prostate cell proliferation *in vitro* and *in vivo* (4,5). A recent epidemiological study showed a decreased risk of symptomatic BPH in men supplemented with vitamin D (6). Significant inverse association between 25OHD levels and prostate volume in adult men included in an Osteoporotic Fractures study was reported by Haghshneno M. et al (4).

It is well known that androgens play a vital role in prostate growth. The most important androgen is testosterone, which is converted to dihydrotestosterone (DHT) inside prostate gland by the local enzyme 5- α reductase (7). DHT stimulates glandular epithelial prostate cell growth and is considered as a major hormonal factor for prostate enlargement in late adulthood.

A widely used marker for prostate gland destruction is the prostate specific antigen (PSA). Elevated serum PSA is observed in men with BPH, prostatitis, or prostate cancer (8). The relationship between 25OHD and testosterone levels is not fully understood and the literature data are sparse and controversial. There is evidence of increase in TT levels after vitamin D supplementation in healthy overweight men (9). Other studies did not find any association between 25OHD and TT in healthy adult men (10) and in healthy male subjects supplemented with vitamin D (11). Our previous study revealed lack of association between 25OHD and PSA in prostate cancer patients (12).

It is widely accepted that 25OHD is the best indicator for vitamin D status (13). A matter of debate in the vitamin D research community is regarded to its reference /target ranges (14). Some authorities indicate that even people without signs or symptoms of pathology are deficient or borderline deficient in vitamin D. There are reports stating that secondary hyperparathyroidism can be corrected with an average 25OHD serum or plasma level at least 75 nmol/L, and a few laboratories use this value as the lower limit of optimal 25OHD serum and plasma concentrations (15,16). The recommended by the World Health Organization serum/plasma level of 50 nmol/L 25OHD for vitamin D deficiency is based on a significant increase of adverse health outcomes related to osteoporotic bone fractures. The lack of consensus on 25OHD target ranges results in substantial variation in the reference ranges or target values used in different research studies.

The seasonal variations in 25OHD levels are well documented (17,18), while for TT the data are limited and controversial. Similar seasonal variations were reported for both 25OHD and TT in adult men with cardiovascular diseases (19,20,21). Other authors found nonsignificant, but similar monthly variations in 25OHD and free testosterone (22). To our knowledge there are no studies examining the causal link between 25OHD and TT levels and their seasonal variations in BPH patients.

The aim of the present study was to evaluate vitamin D status and TT levels in BPH patients and their associations with laboratory parameters for prostate growth, such as PSA.

MATERIALS AND METHODS

Patients

A total of 37 male BPH patients from 52 to 85 years (mean age 67.14 ± 7.77 years) were enrolled in the study. The clinical and laboratory examinations were performed in the Clinic of Urology, "St. Marina" University Hospital, Varna during the period January – December, 2015. All patients were histologically verified, either by systemic transrectal ultrasound-guided tru-cut prostate biopsies (10 cores at least), or by transurethral resection of the prostate (TURP), or open prostatectomy.

Laboratory Examinations

Fasting blood samples were collected only once – at the time of patients' admittance in the Urology Clinic. The serum was separated by centrifugation and all serum samples were frozen and stored at -80°C until analysis.

25OHD

Serum 25OHD was assayed by a validated LC-MS/MS method. The vitamin D status was defined as severe deficiency ($25\text{OHD} < 25\text{nmol/L}$), deficiency ($25\text{OHD} < 50\text{nmol/L}$), sufficiency ($25\text{OHD} > 50\text{nmol/L}$), and optimal values – $25\text{OHD} > 75\text{nmol/L}$.

Biochemical Parameters

Serum levels of glucose, creatinine, calcium, and inorganic phosphate were determined on biochemical analyzer ADVIA 1800, using standard clinical laboratory kits.

Standard chemiluminescent immunometric methods on Immulite 2000 Immunoassay System

(Siemens Healthcare Diagnostics, USA) was used for measuring TT and PSA serum levels.

Other Covariates

Detailed information, regarding patient height, weight, age, and history of the disease was collected by interview at the time of hospitalization. BMI was calculated as $\text{weight (kg)}/\text{height}^2(\text{m}^2)$.

Statistics

GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, California, USA) was used for statistical analysis of continued variables. Student t-test for comparison of means of different parameters was used. The level of significance was set at $p < 0.05$. A non-parametric Spearman correlation analysis was performed to evaluate the associations between serum 25OHD levels and other tested parameters.

The study was approved by the local Ethics Committee, following the guidelines of the Declaration of Helsinki (Protocol No 54/19.05.2016).

RESULTS

Characteristics of the Studied BPH Patients

The characteristics of BPH patients included in the study are presented in Table 1.

The mean serum TT levels of BPH patients were close to the lower limit of 10.4 nmol/L for normal TT according to the recommendations of the Endocrine Society.

The mean level of 25OHD for BPH patients was close to the lower reference limit of 50nmol/L recommended by the US Endocrine Society Guideline (23). Almost half (43.2%) of all studied BPH patients were vitamin D deficient ($25\text{OHD} < 50\text{nmol/L}$). Among them 8.1% were with severe vitamin D deficiency ($25\text{OHD} < 25\text{nmol/L}$). The majority of BPH patients (56.8%) displayed 25OHD levels higher than 50nmol/L . Only 6 patients (16.2%) had optimal 25OHD levels above 75nmol/L .

Overweight and obesity were prevalent in 59.5% of the studied men. No significant difference between 25OHD values of overweight and normal weight BPH patients was established. The other tested laboratory parameters were in the reference range.

Seasonality in 25OHD and TT

Since the 25OHD levels show considerable seasonality, we stratified the studied population by the

Table 1. Baseline characteristics of the studied BPH patients

Parameters	Mean value ± SD	Reference range
Age, years	67.14 ± 7.772	
25OHD, nmol/L	54.98 ± 21.93 *	Optimal: >75 Insufficiency: 50-75 Deficiency: <50
TT, nmol/L	10.74 ± 4.026	Normal: >10.4
PSA, ng/ml	4.152 ± 4.066 *	0-4
Glucose, mmol/L	5.748 ± 1.335	3.3 – 5.8
Creatinine, µmol/L	88.65 ± 19.52	70 – 120
Calcium, mmol/L	2.396 ± 0.1896	2.18 – 2.58
Phosphate, mmol/L	1.000 ± 0.2147	0.80 – 1.50
BMI	26.38 ± 3.178*	Normal <25 Overweight ≥25

TT – total testosterone; BMI – body mass index; PSA – prostate specific antigen; 25OHD – 25-hydroxy vitamin D. * - mean values outside the reference range.

season of blood drawing as follows: cold season (November-May), and warm season (June-October). During the cold period 17 subjects (48.6%) were assayed, and 18 (51.4%) - in the warm season. There were significant differences in 25OHD levels ($p < 0.05$) between the cold and warm season (Fig.1A). During the warm period the 25OHD levels were in the range of vitamin D sufficiency (61.79 ± 19.99 nmol/L), while during the cold season vitamin D deficiency was de-

tected (48.13 ± 16.68 nmol/L). Similar seasonality was not established for TT (Fig. 1B).

Stratification by PSA, TT and 25OHD Levels

Two-thirds of the BPH patients (62.9%) were with PSA values below the upper limit of the reference interval of 4 ng/ml. The remaining 37.1% revealed PSA levels above the reference limit. Patients with abnormal PSA values (>4 ng/ml) were vitamin D deficient (46.12 ± 6.134 nmol/L). Higher 25OHD

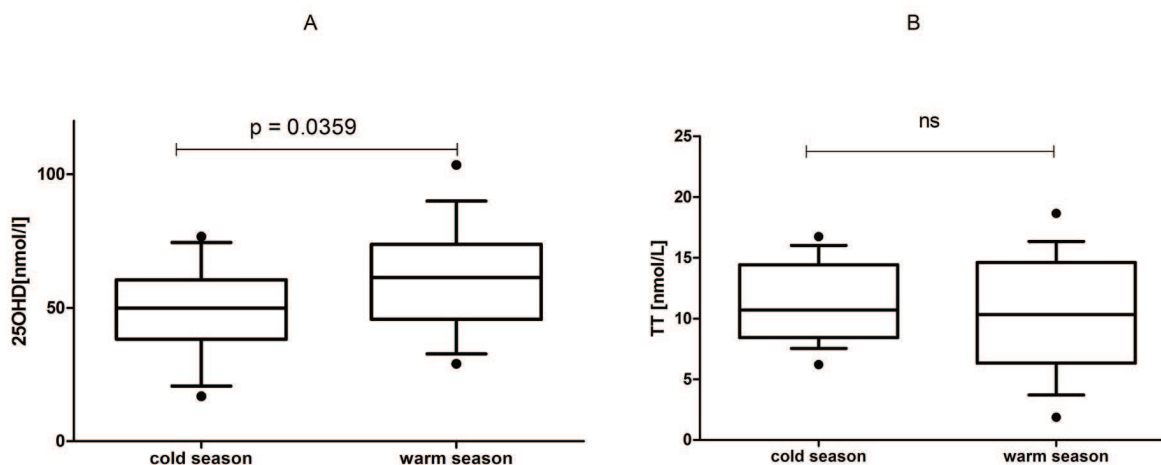


Fig. 1. Seasonal variation of 25OHD and TT levels

(A) 25OHD levels in warm and cold season

(B) TT levels in warm and cold season

Legend: 25OHD – 25-hydroxy vitamin D; TT – total testosterone; ns – non significant

levels (59.21 ± 3.756 nmol/l, $p=0.06$) were established for the group with PSA below the threshold of 4ng/ml (Fig. 2A). For the same PSA groups no differences were found for TT values (Fig. 2B).

threshold for hypogonadism: 56.81 ± 19.30 nmol/L for $TT < 10.4$ nmol/L vs 53.24 ± 24.57 nmol/L for $TT > 10.4$ nmol/L.

The stratification of patients by their 25OHD

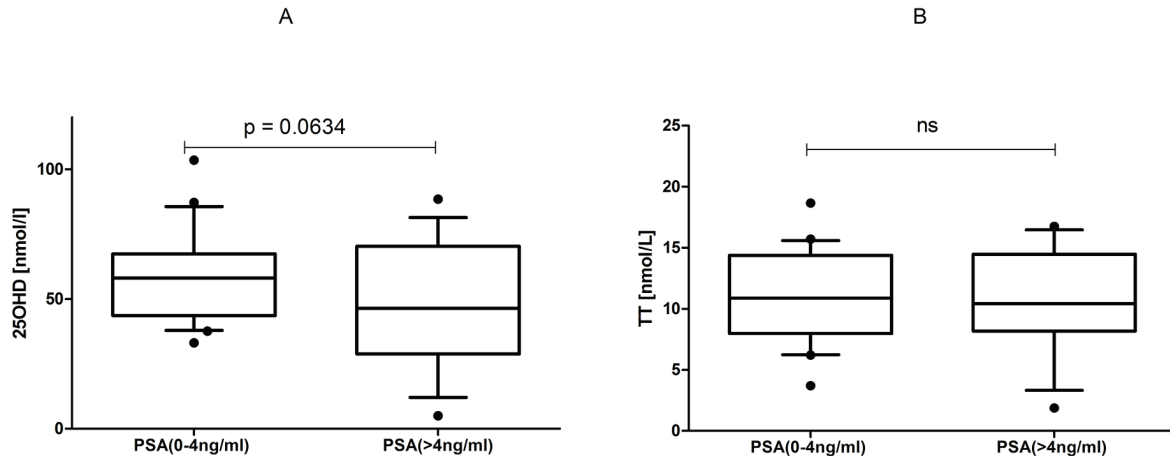


Fig. 2. 25OHD and TT levels in BPH patients stratified by their PSA values

(A) 25OHD in BPH patients according to their PSA values

(B) TT levels in BPH patients according to their PSA values

Legend: 25OHD – 25-hydroxy vitamin D; TT – total testosterone; PSA – prostate specific antigen; ns – non significant

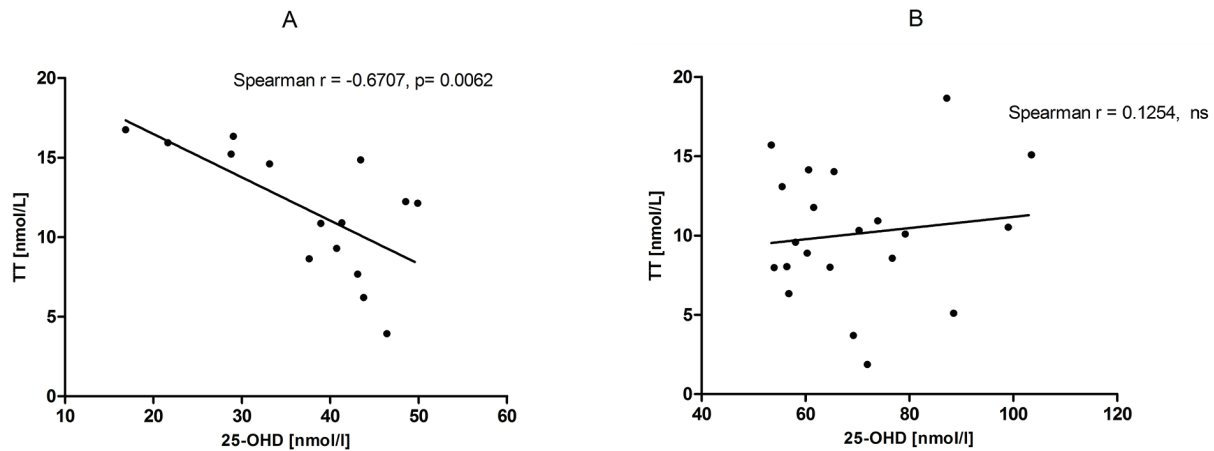


Fig. 3. Association between 25OHD and TT levels in BPH patients

(A) BPH patients with 25OHD below 50 nmol/L

(B) BPH patients with 25OHD above 50 nmol/L

Legend: 25OHD – 25-hydroxy vitamin D; TT – total testosterone

Almost half (51.4%) of BPH patients were with TT levels above the threshold for hypogonadism (10.4nmol/L). No differences in 25OHD levels were detected when the patients were divided by the

levels below and above the cut-off value for vitamin D deficiency revealed no difference in their average TT concentrations (11.55 ± 3.87 nmol/L vs 10.12 ± 4.12 nmol/L).

Associations Between 25OHD and TT

A moderate negative correlation was found between 25OHD and TT (Spearman $r=-0.6707$, $p<0.01$) only for the vitamin D deficient group. In case of vitamin D sufficiency, a weak positive trend was detected (Fig. 3 A,B).

No significant differences in anthropometric and biochemical parameters were found when the BPH patients were stratified by the cut-off for vitamin D deficiency or by the threshold for hypogonadism.

DISCUSSION

Benign prostate hyperplasia (BPH) is one of the most common conditions affecting men (24). It is a chronic progressive disease, histologically proven in approximately 50% of men over 50 years, and 90% of men who are 80 years of age (25). The mechanisms of BPH are multifactorial and not well understood. It is known that androgens play crucial role in prostate growth and differentiation. The increased prostatic concentration of androgens, or increased androgen responsiveness is considered to result in stromal and glandular cell hyperproliferation (26). One of the most important androgens is testosterone, which is converted in the prostate to dihydrotestosterone (DHT). DHT stimulates glandular epithelial prostate cell growth and is considered as a prime factor for prostate enlargement in later adulthood (2).

In our study 51.4% of the studied BPH patients, revealed TT values above the threshold of 10.4nmol/L (mean:13.89±2.335nmol/L). The remaining 48.6% (mean:7.41±2.40nmol/L) fell below the limit of 8nmol/L for hypogonadism, indicated by the European Association of Urology (EAU) and the European Academy of Andrology (EAA) (27). The BPH patients in our study exhibited lowest mean values for TT (10.74nmol/L), compared to those in other studies 13.9nmol/L (28) and 16.75nmol/L (4).

Besides androgens, several other factors such as age, inflammation and diet are considered to play role in BPH (4). The accumulated amount of data reveals that vitamin D is one of the most potent growth regulatory molecules in prostate. Both epithelial and stromal cells express nuclear vitamin D receptor, which binds the active form of vitamin D and initiates a signaling cascade resulting in antiproliferation and prostate growth inhibition (29).

There is no consensus on the optimal 25OHD reference ranges. According to the Endocrine Society, vitamin D deficiency is defined as 25OHD below the “cut off” 50 nmol/L, and vitamin D insufficiency – as a 25OHD between 50–75 nmol/L (23). Many leading authorities believe that health-based reference values are preferable. Accordingly, vitamin D deficiency is defined as 25OHD below 50.0nmol/L, insufficiency: 50.0 – 75.0nmol/L, sufficiency: 75.0-250.0nmol/L, toxicity: >250.0nmol/L (30,31).

The vitamin D status of our BPH patients can be determined as vitamin D insufficiency, showing mean 25OHD value close to the lower limit of the range 50.0 – 75.0nmol/L. Approximately 40% of the studied patients were vitamin D deficient (25OHD < 50nmol/L) and only 16.2% exceeded the optimal limit of 75nmol/L. Our results indicate more severe vitamin D insufficiency when compared to the data of Haghshneno et al, reporting average 25OHD values of 64.37nmol/L, close to the upper reference limit (4). The distribution by 25OHD levels revealed that 83.8% of our BPH patients were below the optimal limit of 75 nmol/L, compared to 69% in the study of Nimpesch et al. The same study reported vitamin D deficiency only in 24% of the studied patients, vs 43% in our study (29). Pitman et al reported similar to our results in a cohort study of urological patients. They found that 52% of their patients were vitamin D deficient (less than 50 nmol/L) (32). Surprisingly, lower median 25OHD values (37.37nmol/L) for the peak season for vitamin D synthesis were reported by Zhang et al for a cohort of 322 men aged 60 to 75 years from South China. More than 71% of their studied patients were vitamin D deficient and only 6.6% fall in the range over 75nmol/L (28).

Vitamin D seasonality is well documented and our results are in agreement with reported data for significantly higher 25OHD levels in summer and fall (warm season) vs lower 25OHD values in spring and winter (cold season) (28,29). The data regarding testosterone seasonal variations are scarce and controversial. Our results did not show seasonal changes in TT levels or seasonal relationship between 25OHD and TT. In agreement with our results, many studies have found no seasonal variation in TT or seasonal relationship between 25OHD and TT (22,29). On the contrary, others have found similar seasonal pattern between 25OHD and TT (19).

PSA is a glycoprotein secreted by the epithelial cells of the prostate gland. PSA is widely used tumor marker for prostate cancer. In addition to prostate cancer, a number of benign conditions, such as BPH, can cause elevation in PSA. It is estimated that every 19% decrease in the PSA levels corresponds to a decrease in a prostate volume by 10% (2). Therefore, most of the studies on BPH patients examine the association between PSA and prostate volume (8,32,33). The median PSA level (3.12ng/ml) of our BPH patients was close to the upper reference limit of 4ng/ml. Consistently with our findings Zhang et al and Putra et al reported similar median PSA values for Indonesian (3.28ng/ml) and Chinese (4.81ng/ml) BPH patients (8,28). In our study, 37.1% of BPH patients revealed PSA levels above the cut-off limit accompanied with vitamin D deficiency. Putra et al reported relatively higher percentage (56.35%) of abnormal PSA for Indonesian BPH patients (8). Although the data concerning the link between PSA and 25OHD levels are very scarce in the literature, Zhang et al established no direct association between serum PSA and 25OHD in patients with and without vitamin D deficiency. They reported strong association between the presence of vitamin D deficiency and BPH, suggesting that vitamin D deficiency may be a marker of BPH (28).

Most studies revealed positive association between TT and 25OHD levels in BPH patients (9,22,29). Surprisingly, we found a weak negative association between 25OHD and TT levels for all studied BPH subjects (Spearman $r = -0.298$, $p = 0.077$). When the patients were stratified into two groups by the cut-off for 25OHD, this negative association becomes stronger and significant (Spearman $r = -0.6707$, $p < 0.01$) for the vitamin D deficient group. The increase of 25OHD levels above 50 nmol/L in the vitamin D sufficient group results in a weak positive trend for elevation also in TT levels. Our data are consistent with the findings of Jorge et al, 2013 reporting no increase in serum TT after high dose vitamin D supplementation (34). It can be speculated that the relationship between these two steroids, 25OHD and TT, somehow depends on the vitamin D status – deficiency or sufficiency.

CONCLUSION

Most of the studied BPH patients were vitamin D insufficient, with 25OHD levels below the optimal limit of 75nmol/L for manifestation of its antiproliferative effects on prostate gland. Higher 25OHD levels were associated with lower PSA values indicating a potential favorable effect of 25OHD on prostate gland.

The contradictory data from mechanistic studies on BPH patients concerning the relationship between 25OHD and TT require further research on their molecular interplay in the prostate gland. Until then, the answer of the question “Is vitamin D associated with testosterone in benign prostate hyperplasia?” will still be pending.

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