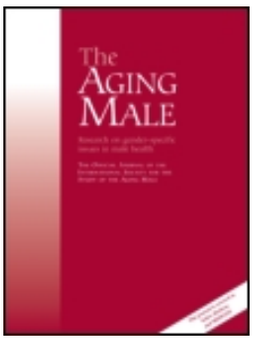

The Aging Male

ISSN: 1368-5538 (Print) 1473-0790 (Online) Journal homepage: http://www.tandfonline.com/loi/itam20

The effect of testosterone treatment on prostate histology and apoptosis in men with late-onset hypogonadism

Ozan Efesoy, Duygu Apa, Mesut Tek & Selahittin Çayan

To cite this article: Ozan Efesoy, Duygu Apa, Mesut Tek & Selahittin Çayan (2016) The effect of testosterone treatment on prostate histology and apoptosis in men with late-onset hypogonadism, The Aging Male, 19:2, 79-84, DOI: 10.3109/13685538.2016.1148131

To link to this article: http://dx.doi.org/10.3109/13685538.2016.1148131

The Aging Male

http://informahealthcare.com/tam
ISSN: 1368-5538 (print), 1473-0790 (electronic)

Aging Male, 2016; 19(2): 79–84
 DOI: 10.3109/13685538.2016.1148131

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The effect of testosterone treatment on prostate histology and apoptosis in men with late-onset hypogonadism

Ozan Efesoy[1], Duygu Apa[2], Mesut Tek[1], and Selahittin Çayan[1]

[1]*Department of Urology, University of Mersin School of Medicine, Mersin, Turkey and* [2]*Department of Pathology, University of Mersin School of Medicine, Mersin, Turkey*

Abstract

Objectives: To investigate the effect of testosterone replacement therapy (TRT) on prostate histology and apoptosis in men with late-onset hypogonadism (LOH).
Methods: The study included 25 men, having LOH with prostate-specific antigen (PSA) level of 4 ng/ml or less. All patients underwent transrectal ultrasound guided prostate biopsy at baseline, and received testosterone undecanoate treatment for 1 year. Prostate biopsy was repeated at the end of 1 year of testosterone therapy. In addition to clinical and biochemical parameters, prostate histology and apoptotic index (AI) were compared before and after the TRT.
Results: The mean serum total testosterone significantly increased from 178.04 ± 51.92 to 496.28 ± 103.73 ng/dl ($p = 0.001$). No significant differences were observed in serum total and free PSA level, prostate volume and maximal urinary flow rate. There were also no significant differences in AI, stroma/epithelial cells ratio, Ki-67 positive cells and atrophy score of prostate tissue before and after the TRT.
Conclusions: This study demonstrated that TRT did not affect serum PSA level, prostate volume and maximal urinary flow rate. This study also suggests that TRT does not cause the risk for prostate cancer development, because of no significant differences in prostate histology after TRT.

Keywords

Testosterone, prostate cancer, prostate volume, LUTS, AMS, IIEF

History

Received 7 January 2016
Revised 26 January 2016
Accepted 26 January 2016
Published online 26 February 2016

Introduction

Late-onset hypogonadism (LOH) is defined as ''a clinical and biochemical syndrome associated with advancing age and characterized by symptoms and a deficiency in serum testosterone levels (below the young healthy adult male reference range)'', by the ISA, ISSAM, EAU, EAA and ASA [1]. This condition may result in significant detriment to quality of life, and adversely affect the function of multiple organ systems [2]. Clinical findings of the LOH may be improved with testosterone r*eplacement* therapy (TRT). In addition to detrimental effect of sexual functions, men with LOH have increased waist circumference, hyperglycemia, hypertriglyceridemia, hyperlipidemia and a history of diabetes [3]. TRT also improves body weight and metabolic factors in men with LOH, and withdrawal of testosterone treatment reverses these beneficial effects [4]. Tajar et al. [5] reported that the incidence of LOH was 2.1% in the subjects aged 40–79 years in the European Male Aging Study. Various studies suggest that the annual incidence of LOH in USA is as high as 500 000 new cases in men aged 40–69 years [6]. This incidence and number of men with LOH who need TRT will increase, because of rising in older age population in the World [5].

In their study, conducted in 261 hypogonadal elderly men with erectile dysfunction, Yassin et al. [7] pointed out long term testosterone treatment reduces obesity parameters and improves metabolic syndrome and health-related quality of life. Major concerns of clinicians who treat LOH would be development of prostate cancer due to high serum testosterone level, and exogenous testosterone may reverse occult prostate cancer to clinical prostate cancer. This concern has been supported since 1941 by Huggins and Hodges with a study showing a decrease in serum phosphatase after castration, and again increase after the testosterone treatment [8]. However, although it is well known that prostate is an androgen dependent organ, all androgen receptors are saturated and a further increase in circulating levels of testosterone has no effect on the prostate [9], and role of androgens on the development of prostate cancer has not been highlighted with clinical and histologic studies.

The aim of this study was to investigate the effect of TRT on prostate histology and apoptosis in men with LOH. We also investigated the effect of TRT on total and free

Address for correspondence: Selahittin Çayan, MD, FECSM, Professor of Urology, Department of Urology, School of Medicine, University of Mersin, 33033 Mersin, Turkey. Fax: +90 324 337 4311. E-mail: selcayan@mersin.edu.tr

prostate-specific antigen (PSA) levels, prostate volume and maximal urinary flow rate in this patient population.

Methods

The study was carried out between March 2008 and December 2011 at the University of Mersin School of Medicine Hospital. The study design was approved by the local ethics committee, consisting of the members from the Turkish Ministry of Health and University of Mersin School of Medicine. An inform consent was taken from all patients included in the study.

At present, as shown in Figure 1, the study included 52 patients, met clinical and biochemical LOH with serum total testosterone level of <200 ng/dl. Of the patients, 15 were excluded from the study due to serum PSA level of >4.0 ng/ml, severe lower urinary tract symptoms (LUTS), having prostate treatment (medical or surgical), use of any drugs that might affect hypothalamic-pituitary tract, suspected or confirmed prostate or male breast cancer, polycythemia, congestive heart failure and obstructive sleep apnea syndrome. Of the patients, six did not want to be involved in the study after taking the inform consent. Therefore, the study population consisted of 31 consecutive men having LOH with PSA level of 4 ng/ml or less. All patients underwent transrectal ultrasound guided prostate biopsy at baseline. Of the patients, four were excluded due to prostate cancer in two, atypical small acinar proliferation in one and prostatic intraepithelial neoplasia in one. Of the patients, 27 received 1 year of testosterone undecanoate treatment (Nebido®, Bayer Schering Pharma AG, Germany). Testosterone undecanoate treatment was repeated after 6 weeks of the first injection, and then every 3 month for 1 year (totally five injections for 1 year).

After initial evaluation and clinical diagnosis of LOH, all patients had clinical and biochemical evaluation every 3 month under the treatment. TRT was stopped in two patients due to polycythemia (Hct >54%) in one patient at 6 month, and increase in PSA level (>4 ng/nl) in one patient at 9 month of the treatment.

Finally, of the patients who received TRT, 25 completed 1 year of therapy and prostate biopsy was repeated. Prostate histology and apoptotic index (AI) were compared before and after the TRT. In addition, total and free PSA levels, prostate volume, maximal urinary flow rates, Aging Male Symptom (AMS) Scale, International Index of Erectile Function (IIEF-5) and International Prostate Symptom Score (IPSS) were compared from pretreatment to post-treatment at 1 year.

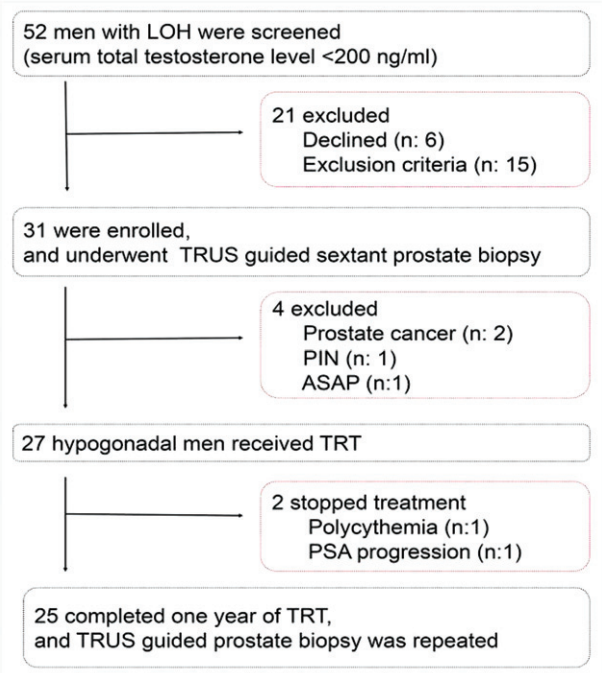

Figure 1. Follow-up of the participants through the study.

Questionnaires to assess clinical findings

All patients were assessed using validated questionnaires in Turkish: AMS scale for presence of hypogonadism, IIEF-5 for erectile functions, IPSS to assess LUTS [10].

Clinical prostate testing

Peak flow rate (Q_{max} as ml/s) was measured during uroflowmetric study with a minimum of 150 ml urination volume. Prostate volume was measured with a transrectal ultrasound during the prostate biopsy at the beginning and end of the study. During the 3-month periodical controls, transabdominal ultrasound was repeated.

Biochemical and hormonal testing

After a 12–14 h fast, between 07.00 a.m. and 11.00 a.m., we took venous blood samples. The automated cell counter was used to determine complete blood count. Testosterone level was determined by mass spectroscopy. Triglycerides, total cholesterol and HDL-cholesterol were quantified by standard enzymatic-colorimetric methods. LDL-cholesterol was determined by the Friedewald formula. Total and free PSA levels were measured using electro-chemoluminescence immunoassay method.

Prostate biopsy

All patients received cleansing enema before the biopsy. The participants received 500 mg of oral ciprofloxacin 1 h before the biopsy, and it was given twice daily for 3 days. Following evaluation of the prostatic anatomy while the patient in the left lateral decubitus position, and periprostatic nerve blockade performed using 10 ml of 2% lidocaine, classic sextant biopsy specimens were obtained. All of these procedures were performed under the guidance of standard gray-scale ultrasound, and 7.5 MHz rectal probe using 18 Gauge biopsy needle, and automatic biopsy gun. Distal ends of all biopsy specimens were marked with an ink, and sent to the pathology laboratory in individually numbered tubes.

Histopathologic evaluation

Histopathological and *immunohistochemistry* examinations were performed to each case by one of the authors (D.D.A.) before and after treatment. Stroma-epithelial ratio was calculated for each area by dividing percent stroma by percent epithelium after stroma-to-epithelial image analysis technique as described [11,12]. Atrophy score was calculated, i.e. the number of atrophic glands as a percentage of the total number of glands. The cell proliferation rate by using Ki-67/MIB-1

antibody was also calculated. Cells staining positive were expressed as the number of stained cells per 100 epithelial cells.

Apoptosis was detected by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labelling (TUNEL) assay using a (TdT)-FragEl DNA fragmentation detection kit (Oncogene, Cambridge, MA). This *in situ* apoptosis kit allows recognition of apoptotic nuclei in paraffin-embedded tissue sections by fragment end labeling of DNA. In this assay, TdT binds to exposed 30-OH ends of DNA fragments generated in response to apoptotic signals and catalyzes the addition of biotinlabeled and unlabeled deoxynucleotides. Biotinylated nucleotides are detected using streptavidin–horseradish peroxidase conjugate. Diaminobenzidine reacts with the labelled sample to generate an insoluble brown-coloured complex at the site of DNA fragmentation. Counter staining with methyl green aids in the morphological evaluation and characterization of normal and apoptotic cells [13]. AI was calculated, i.e. the number of apoptotic cells as a percentage of the total number of cells (Figure 2).

Statistical analyses

Comparisons from the baseline to the end of the therapy were done with the Paired Samples t test and Wilcoxon test according to distribution of data. Data were summarized as mean ± standard deviation or median [interquartile range]. Statistical analyses were performed using PASW v.18 statistical package (SPSS Inc., Chicago, IL), and p values <0.05 were accepted as statistically significant.

Results

The mean age of the patients was 58.08 ± 5.34 years (range 42–70). Table 1 shows clinical and biochemical parameters of the patients at baseline and at the end of the treatment. The mean AMS total score significantly increased from 60.64 ± 10.13 to 26.16 ± 5.87 ($p = 0.001$). Sub-domains including psychological ($p = 0.001$), somatic ($p = 0.001$) and sexual ($p = 0.001$) domains also significantly increased. The mean IIEF-5 total score significantly increased from

Table 1. Results of clinical and biochemical parameters at the baseline and end of the treatment.

	Baseline	End of the treatment	p values
AMS scale total score	60.64 ± 10.13	26.16 ± 5.87	0.001
Psychological domain	17.64 ± 2.44	8.20 ± 1.89	0.001
Somatic domain	25.24 ± 5.24	10.52 ± 3.02	0.001
Sexual domain	18.56 ± 3.69	7.36 ± 2.13	0.001
IIEF-5 total score	11.12 ± 3.65	19.84 ± 3.74	0.001
Total testosterone (ng/dl)	178.04 ± 51.92	496.28 ± 103.73	0.001
IPSS total score	9.44 ± 2.81	10.04 ± 2.96	0.061
Q_{max} (ml/sn)	18.16 ± 3.71	17.88 ± 4.33	0.589
Prostate volume (ml)	28.28 ± 14.00	29.72 ± 13.78	0.150
Total PSA (ng/ml)	2.08 ± 0.89	2.20 ± 0.95	0.101
Free PSA (ng/ml)	0.65 ± 0.29	0.67 ± 0.30	0.297
Total cholesterol (mg/dl)	208.70 ± 40.37	218.04 ± 45.28	0.201
HDL cholesterol (mg/dl)	41.58 ± 9.15	44.54 ± 8.71	0.061
LDL cholesterol (mg/dl)	127.19 ± 32.30	129.10 ± 33.83	0.761
Triglyceride (mg/dl)	184.25 ± 82.71	200.92 ± 91.41	0.186
Hemoglobin (g/dl)	14.66 ± 1.43	15.64 ± 1.13	0.001
Hematocrit (%)	42.92 ± 4.10	46.31 ± 3.97	0.001

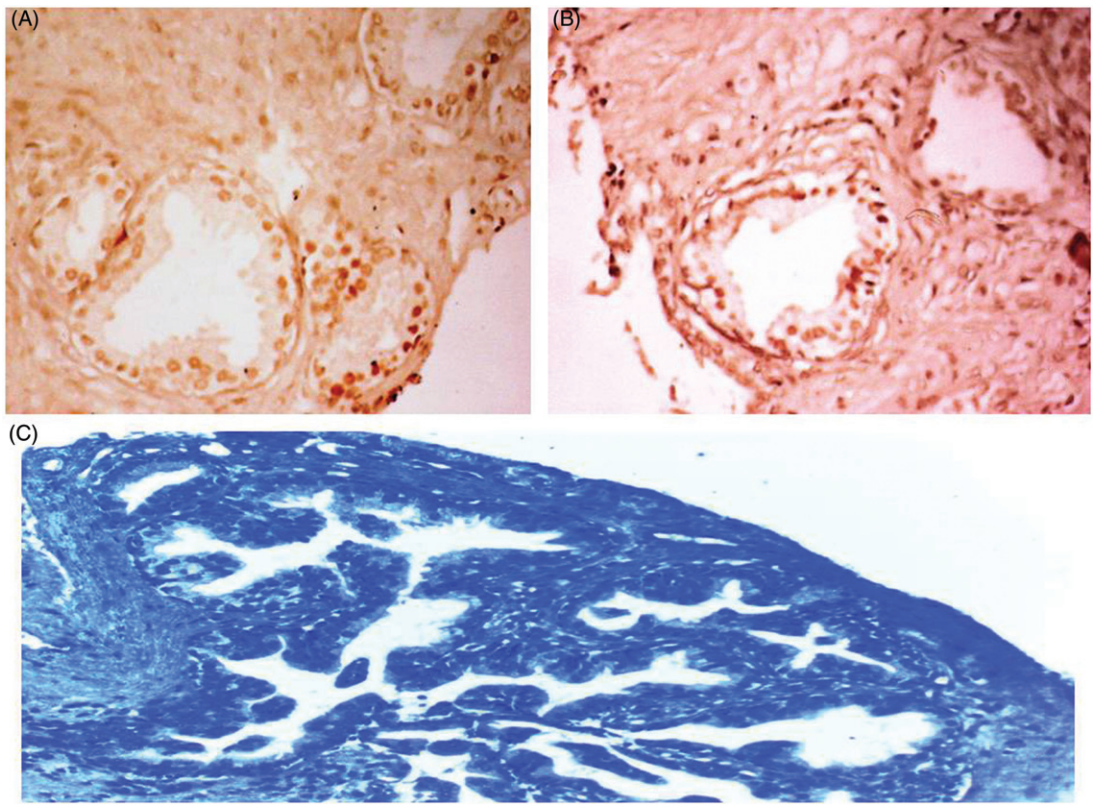

Figure 2. Histological findings show focal tunnel staining in prostatic gland epithelium before and after the treatment. (A) The immunohistochemical staining of the nuclei of prostatic gland epithelium with tunnel before the treatment (tunnel, ×200). (B) The immunohistochemical staining of the nuclei of prostatic gland epithelium with tunnel after the treatment (tunnel, ×200). (C) Cell proliferation in prostatic gland epithelium by Ki-67/MIB-1 antibody.

11.12 ± 3.65 to 19.84 ± 3.74 ($p = 0.001$). The mean serum total testosterone significantly increased from 178.04 ± 51.92 to 496.28 ± 103.73 ng/dl ($p = 0.001$). No significant differences were observed in IPSS total score ($p = 0.061$), serum total ($p = 0.101$) and free ($p = 0.297$) PSA levels, prostate volume ($p = 0.150$) and maximal urinary flow rate ($p = 589$). Mean hemoglobin and hematocrit levels statistically significantly increased ($p = 0.001$ and $p = 0.001$, respectively), but this increase was not clinical meaningful. No significant differences were observed in total cholesterol ($p = 0.201$), HDL cholesterol ($p = 0.06$), LDL cholesterol ($p = 0.761$) and triglyceride ($p = 0.186$).

Table 2 shows histopathological and *immunohistochemistry* results of the patients at baseline and at the end of the treatment. There were no significant differences in AI ($p = 0.076$), stroma/epithelial cells ratio ($p = 0.117$), Ki-67 positive cells ($p = 0.242$) and atrophy score of peripheral zone ($p = 0.07$) before and after the TRT. Figure 2 shows prostate histology and apoptosis in a patient before and after the treatment.

Discussion

Several studies have suggested that TRT may improve LUTS and voiding functions [10,14–17]. Yassin et al. [15] investigated long term TRT and TRT withdrawal on obesity and prostate-related parameters in 262 hypogonadal men. Of the patients, 147 had TRT interrupted, and 115 patients were treated continuously. Prior to interruption, TRT resulted in improvements in residual urine volume, bladder wall thickness, AMS, IIEF-EF, IPSS and obesity parameters while PSA and prostate volume increased. TRT interruption resulted in worsening of the symptoms [15].

Hypogonadal men may need life-long TRT. However, clinicians who treat hypogonadal men with TRT have major concerns about development of prostate cancer and cardiovascular risks in this population [2]. Clinical studies have not supported role of androgens on the development of prostate cancer [2,9,15]. Serum total testosterone is in the lowest level in the life term 6th decade and later, while the incidence of prostate cancer is in the highest level. In addition, prostate cancer is very rarely seen in the 2nd and 3rd decades of men in whom serum testosterone level at the highest level [6]. Some case presentations on the occurrence of prostate cancer in men with bilateral anorchia or bilateral testicular atrophy have made the theory ''prostate cancer is never seen in castrated men'' unproved [18,19]. In addition, prevalence of prostate cancer in hypogonadal men is similar to normogonadal men in the PCPt studies [20,21].

In the collaborative analysis of 18 prospective studies, no associations were found between the risk of prostate cancer and serum concentrations of testosterone, calculated free testosterone, dihydrotestosterone, dehydroepiandrosterone sulfate, androstenedione, androstanediol glucuronide, estradiol or calculated free estradiol [22]. Prostate growth is exquisitely sensitive to variations in androgen concentrations at very low concentrations, but becomes insensitive to changes in androgen concentrations at higher levels. This pattern is consistent with the observation that androgens exert their prostatic effects primarily via binding to the androgen receptor (AR), and that maximal androgen-AR binding is achieved at serum testosterone concentrations well below the physiologic range. A saturation model is proposed that accounts for the seemingly contradictory results in human prostate cancer studies. Changes in serum testosterone concentrations below the point of maximal androgen-AR binding will elicit substantial changes in prostate cancer growth, as seen with castration, or with testosterone administration to previously castrated men. In contrast, once maximal androgen-AR binding is reached the presence of additional androgen produces little further effect [23]. In conclusion, high endogenous serum testosterone does not increase the risk of developing prostate cancer, and low serum testosterone does not protect against prostate cancer [3].

In addition to saturation theory, current studies similarly fail to indicate any increased risk of prostate cancer in men receiving testosterone therapy. Calof et al. [24] performed a meta-analysis of 19 randomized clinical trials to determine the risks of adverse events associated with testosterone replacement in older men, and they found that rates of prostate cancer, PSA >4 ng/ml, and prostate biopsies were numerically higher in the testosterone group than in the placebo group, although differences between the groups were not individually statistically significant. Shabsigh et al. [25] reported a systemic review of 44 clinical studies, and they found prostate cancer in 1.3% of hypogonadal men receiving TRT and in 1.5% of placebo group. Feneley and Carruthers included 1365 men with symptomatic androgen deficiency and receiving TRT, and found 14 new cases (1.3%) of PCa with a mean 6.3-year follow-up period [26]. In a very recent prospective three parallel studies, Haider et al. [27] found diagnosis of new prostate cancer in 11 cases of 1023 hypogonadal men receiving TRT with a median of 5-year follow-up duration. The authors reported that incidence of prostate cancer was even lower in their study population than in the PLCO and ERSPC long-term screening trials (7.35% and 9.6%, respectively). In our study, we found no significant differences in serum total and free PSA levels, prostate volume and maximal urinary flow rate. In addition, none developed prostate cancer after the 1-year testosterone treatment.

Prostate is an androgen dependent organ, however, role of testosterone on the molecular mechanisms and pathophysiology

Table 2. Histopathological and *immunohistochemistry* results of the patients at baseline and at the end of the treatment.

	Baseline	End of the treatment	*p* values
Stroma-epithelial ratio	18.36 ± 8.65	15.72 ± 6.98	0.117
Apoptotic index, % of glands	2 [1–4.5]	4 [1–10]	0.076
Atrophy score, % of glands	10 [4.5–14]	5 [3–9.5]	0.070
Ki-67 (MIB1), % of positive cells	0 [0–0.33]	0.33 [0–0.66]	0.242

of prostate diseases has not been exactly highlighted. Interactions between epithelium and stroma are known to be important already during prostate development and this interplay is critical also in development, progression of primary tumours and growth of metastases. Prostate cancer is an adenocancer, and mostly originated from epithelium of peripheral zone. However, recent studies suggest that stromal mediators have played important role to develop and aggressiveness of prostate cancer. It is therefore reasonable to expect that future biomarkers and therapeutic targets can be identified in the prostate tumor and metastasis stroma and this possibility should be further explored [28]. The stromal/epithelial ratio was determined as 1.2 in young men with non-hyperplasia prostate, while it was 4.6 in symptomatic men with benign prostatic hyperplasia (BPH) [12]. Marks et al. [29] reported that the stroma/epithelial ratio increased from 3.2 to 17.4 in men with symptomatic BPH receiving finasteride, 5-alpha reductase inhibitor, after 30 month of treatment. In our study, although stroma-epithelial ratio was high, because of low serum testosterone level of the hypogonadal men at the beginning of the study, this rate decreased over time with the TRT. However, this decrease was statistically insignificant.

In a study, apoptotic cells were found to be statistically lowered in the prostate cancer group, compared with the benign prostate hyperplasia group, suggesting that although the triggering of these changes is unknown, proliferative atrophy due to inflammation, ischemia and oxidative stress is related to prostatic carcinoma development [30]. Androgens may play significant role in the balance between prostatic cell proliferation, apoptosis and atrophy. Interactions between epithelium and stroma are known to be important already during prostate development and this interplay is critical also in development, progression of primary tumors and growth of metastases [28]. Marks et al. [31] investigated the effects of TRT on prostate tissue of 44 aging men with low serum testosterone level. The treatment group received 150 mg of testosterone enanthate, and the placebo group received only saline injection intramuscularly every 2 weeks for 6 month. After the 6-month treatment, no treatment-related change was observed in prostate histology, tissue biomarkers (androgen receptor, Ki-67 and CD34), gene expression (including AR, PSA, PAP2A, VEGF, NXK3 and clusterin), or cancer incidence or severity. Their preliminary data suggest that in aging men with LOH, 6 month of TRT normalizes serum androgen levels but appears to have little effect on prostate tissue androgen levels and cellular functions. In our study, we found no significant differences in AI, stroma/epithelial cells ratio, Ki-67 positive cells and atrophy score of peripheral zone before and after 1 year of TRT, furthermore slightly decrease in gland atrophy score and slightly increase in Ki-67 positive cells and apoptotic cells after the treatment.

Conclusions

This study demonstrated that TRT did not affect serum PSA level, prostate volume and maximal urinary flow rate. This study also suggests that TRT does not cause the risk for prostate cancer development, because of no significant differences in prostate histology after TRT. Although 1 year of testosterone therapy is safe to determine prostate cancer, further studies with larger number of patients are needed.

Declaration of interest

The authors report no declarations of interest.

References

1. Wang C, Nieschlag E, Swerdloff R, et al. Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA, and ASA recommendations. Eur Urol 2009;55:121–30.
2. Morgentaler A, Zitzmann M, Traish AM, Fox A. Inetrnational expert consensus conference on testosterone deficiency and its treatment held in Prague, Czech Republic. Aging Male 2015;18: 205–6.
3. Yassin AA, Nettleship JE, Almehmadi Y, et al. Is there a relationship between the severity of erectile dysfunction and the comorbidity profile in men with late onset hypogonadism. Arab J Urol 2015;13:162–8.
4. Yassin A, Almehmadi Y, Saad F, et al. Effects of intermission and resumption of long-term testosterone replacement therapy on body weight and metabolic parameters in hypogonadal in middle-aged and elderly men. Clin Endocrinol (Oxf) 2016;84:107–14.
5. Tajar A, Huhtaniemi IT, O'Neill TW, et al. Characteristics of androgen deficiency in late-onset hypogonadism: results from the European Male Aging Study (EMAS). J Clin Endocrinol Metab 2012;97:1508–16.
6. Isbarn H, Pinthus JH, Marks LS, et al. Testosterone and prostate cancer: revisiting old paradigms. Eur Urol 2009;56:48–56.
7. Yassin DJ, Doros G, Hammerer PG, Yassin AA. Long-term testosterone treatment in elderly men with hypogonadism and erectile dysfunction reduces obesity parameters and improves metabolic syndrome and health-related quality of life. J Sex Med 2014;11:1567–76.
8. Huggins C, Hodges CV. Studies on prostatic cancer: I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate 1941. J Urol 2002;168:9–12.
9. Saad F, Yassin AA, Haider A, Gooren L. Effects of testosterone on the lower urinary tract go beyond the prostate: new insights, new treatment options. Arab J Urol 2011;9:147–52.
10. Karazindiyanoğlu S, Çayan S. The effect of testosterone therapy on lower urinary tract symptoms/bladder and sexual functions in men with symptomatic late-onset hypogonadism. Aging Male 2008;11: 146–9.
11. Veltri RW, Park J, Miller MC, et al. Stromal-epithelial measurements of prostate cancer in native Japanese and Japanese-American men. Prostate Cancer Prostatic Dis 2004;7:232–7.
12. Shapiro E, Becich MJ, Hartanto V, et al. The relative proportion of stromal and epithelial hyperplasia is related to the development of symptomatic benign prostate hyperplasia. J Urol 1992;147:1293–7.
13. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493–501.
14. Kalinchenko S, Vishnevskiy EL, Koval AN, et al. Beneficial effects of testosterone administration on symptoms of the lower urinary tract in men with late-onset hypogonadism: a pilot study. Aging Male 2008;11:57–61.
15. Yassin A, Nettleship JE, Talib RA, et al. Effects of testosterone replacement therapy withdrawal and re-treatment in hypogonadal elderly men upon obesity, voiding function and prostate safety parameters. Aging Male 2016;8:1–6.
16. Shigehara K, Sugimoto K, Konaka H, et al. Androgen replacement therapy contributes to improving lower urinary tract symptoms in patients with hypogonadism and benign prostate hypertrophy: a randomized controlled study. Aging Male 2011;14:53–8.
17. Nakamura M, Fujimura T, Nagata M, et al. Association between lower urinary tract symptoms and sexual dysfunction assessed using the core lower urinary tract symptom score and international index of erectile function-5 questionnaires. Aging Male 2012;15: 111–14.
18. Sharkey DA, Fisher ER. Carcinoma of the prostate in the absence of testicular tissue. J Urol 1960;83:468–70.
19. Daniell HW, Tam EW. Testicular atrophy in therapeutic orchiectomy specimens from men with prostate carcinoma: association with prior prostate bed radiation and older age. Cancer 1998;83:1174–9.

20. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level or =4.0 ng per milliliter. N Engl J Med 2004;350:2239–46.
21. Morgentaler A, Rhoden EL. Prevalence of prostate cancer among hypogonadal men with prostate-specific antigen levels of 4.0 ng/mL or less. Urology 2006;68:1263–7.
22. Roddam AW, Allen NE. Endogenous Hormones and Prostate Cancer Collaborative Group, et al. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008;100:170–83.
23. Morgentaler A, Traish AM. Shifting the paradigm of testosterone and prostate cancer: the saturation model and the limits of androgen-dependent growth. Eur Urol 2009;55:310–20.
24. Calof OM, Singh AB, Lee ML, et al. Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. J Gerontol Biol Sci Med Sci 2005;60:1451–7.
25. Shabsigh R, Crawford ED, Nehra A, et al. Testosterone therapy in hypogonadal men and potential prostate cancer risk: a systematic review. Int J Impot Res 2009;21:9–23.
26. Feneley MR, Carruthers M. Is testosterone treatment good for the prostate? Study of safety during long-term treatment. J Sex Med 2012;9:2138–49.
27. Haider A, Zitzmann M, Doros G, et al. Incidence of prostate cancer in hypogonadal men receiving testosterone therapy: observations from 5-year median follow-up of 3 registries. J Urol 2015;193: 80–6.
28. Hägglöf C, Bergh A. The stroma-a key regulator in prostate function and malignancy. Cancers 2012;4:531–48.
29. Marks LS, Partin AW, Dorey FJ, et al. Long-term effects of finasteride on prostate tissue composition. Urology 1999;53: 574–80.
30. Kosova F, Temeltas G, Arı Z, Lekili M. Possible relations between oxidative damage and apoptosis in benign prostate hyperplasia and prostate cancer patients. Tumour Biol 2014;35: 4295–9.
31. Marks LS, Mazer NA, Mostaghel E, et al. Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. JAMA 2006;296: 2351–61.