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Daily subcutaneous testosterone for management of testosterone deficiency

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1. ABSTRACT

Testosterone deficiency (TD) is a public health concern, a predictor of metabolic syndrome, and is associated with an increased all-cause and cardiovascular mortality. Testosterone deficiency in men is treated by a variety of methods including injectable testosterone compounds, patches, gels, pellets, and oral preparations. The use of testosterone alone has been linked to various adverse effects including, infertility, testicular atrophy, erythropoiesis, and gynecomastia. To determine the effectiveness of therapy using the Daily Subcutaneous Testosterone (DST) method in combination with human chorionic gonadotropin (hCG) and an aromatase inhibitor (anastrozole), a retrospective analysis was conducted of men diagnosed and treated for TD. Changes in testosterone, estradiol, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating hormone (FSH), dihydrotestosterone (DHT), dehydroepiandrosterone sulfate (DHEA-S), prostate-specific antigen (PSA), pregnenolone, and hemoglobin were determined. There was a significant increase in total testosterone, free serum testosterone, and direct free testosterone in the testosterone treated patients. There was a significant increase in total and free testosterone levels with the DST method combined with hCG and anastrozole,suggesting that DST therapy is a viable option to restore testosterone levels in men.

2. INTRODUCTION

2.1. Definition of testosterone deficiency

Testosterone is produced by the gonads of men and women. In men, the Leydig cells of the testes produce testosterone, and in women it is produced in the ovaries. Smaller amounts of testosterone are produced in the adrenal glands of both sexes. Testosterone production typically decreases with age, and this decrease may be accompanied by various health effects and a compromised quality of life. Although both sexes can be affected by low testosterone levels, the phenomenon is more extensively studied in men. Worldwide, the prevalence of subnormal free testosterone levels in lean, overweight, and obese non-diabetic men was found to be 26%, 29% and 40%, respectively (1).

Testosterone deficiency is defined as low serum testosterone and is associated with various symptoms such as a reduction in libido, erectile dysfunction, fatigue, difficulties in concentration, sleep disturbance, decreased muscle mass and bone density and increased body fat (2). Testosterone deficiency is associated with a number of health complications including osteoporosis, cardiovascular disease, type 2 diabetes, and metabolic syndrome, among others (3-8). The effects of low testosterone can vary between individuals but have patterns that correspond to the age that the deficiency occurs (2). In a boy during puberty, low testosterone may be associated with slower than

normal growth. In the case of hypogonadism of young boys, there may be a failure of the development of secondary sex characteristics (9). Low testosterone in mature men can be characterized by a decrease in muscle mass, hair loss, and reduction in mean body mass (2). The age-related decrease in testosterone is often referred to as andropause or male menopause, terms not favored by nationally recognized societies, therefore must be used with some reserve.

2.2. Diagnosing and treatment approaches to testosterone deficiency

Determination of testosterone levels are ascertained by measuring serum total testosterone, free testosterone, bioavailable testosterone, and free androgen index. To date, calculation of free serum testosterone by measurement of total testosterone and sex hormone binding globulin (SHBG) provide more accuracy, and is the method most commonly utilized by laboratories (10, 11). Testosterone levels of less than 300 ng/dL in men are considered to be low (12).

However, the absolute value of testosterone level to determine TD has come in to question in recent years due to differences among ethnicity, geographic location, intra-individual variability, as well as variations in laboratory testing (13). Furthermore, clinical signs of TD may appear in men before laboratory testosterone levels decline to less than 300 ng/dL. Wang *et al*. indicate that total testosterone levels that are less than 346 ng/dL or free serum testosterone levels less 72 pg/mL are considered to be abnormal, a threshold to consider treatment with testosterone therapy (13).

According to the 2016 Testosterone Consensus Resolution, testosterone therapy is effective, rational, and evidence based (14). Low testosterone in men is treated by using a number of methods. These include application of testosterone via intramuscular or subdermal injections, patches, gels, pellets, oral preparations, and using testosterone alone or in combination with other hormones and non-hormone molecules (15). Intramuscular (IM) injectable forms include testosterone enanthate, testosterone propionate, testosterone cypionate, and testosterone undecanoate. Injectable testosterone undecanoate is a newer treatment form that can be given less frequently than older testosterone treatment forms and is thought to be associated with less adverse effects (16, 17). However, a low incidence of pulmonary oil microembolization (POME) reaction has been reported during or immediately after administration by medical personnel, greatly limiting its availability in clinical practice (18). Oral forms of testosterone undecanoate are also available and provided with lipophilic solvents to prevent first-pass inactivation and liver toxicity (19).

In general, IM testosterone injections are administered by medical personnel in a clinic setting on a weekly or biweekly interval. The advantages of injectable testosterone are achievement of reliable serum peak levels, and low cost. Infrequent injections however, result in large variability of peak-trough blood testosterone levels and are also associated with fluctuations in mood and libido (20). Similarly, large fluctuations in other hormones such as serum estradiol and DHT are found with infrequent, large IM injections (21). A third of patients experience pain and bleeding with deep intramuscular injection (20).

Daily gels, patches, pellets, or oral testosterone dosing is described (22). Pharmacological comparisons of various testosterone treatment formulations have been examined (23, 24). Daily administration of testosterone in gel form provides a more stable blood concentration of testosterone than weekly IM injections (25). The downside to topical application includes a risk of passive transference of hormone to family members causing virilization of children,a black box warning attached by the FDA. Topical administration is also associated with increased PSA levels and induction of acne through conversion of testosterone to dihydrotestosterone (DHT) via 5 alpha-reductase enzyme in the skin (26). In a significant portion of patients, skin irritation limits continuation of use, and cost is prohibitive as generic versions are currently not available in the US (27).

Oral administration is not commonly used in the U.S. due to the significant first pass effect and the adverse effects of formulations designed to bypass this effect (22). Lesser used routes of administration, such as buccal and nasal forms, also result in less stable blood testosterone levels and lead to a number of effects including site irritation and inflammation (22). Subcutaneous administration has been used in the form of specially formulated pellets or with small needle injections. With pellets, a medical provider surgically implants one every 3 or 6 months. Serum testosterone levels peak within a month of implant and can last for 3 or more months. Although this method encourages patient compliance, it is associated with a number of adverse effects. There is a moderately high incidence of pellet extrusion (10%), and a number of clinical effects such as site bleeding, infections, and fibrosis are not uncommon. Pellet implantation is also more invasive since application and removal (if medically necessary) requires surgical incision with local anesthetic (22).

Kaminetsky *et al.* studied the pharmacokinetics of weekly subcutaneous testosterone compared to intramuscular testosterone dosing (24). Both 50 and 100 mg subcutaneous doses of testosterone enanthate (TE) administered with an autoinjector weekly for 6 weeks were well tolerated and achieved normal

average concentrations of serum testosterone within 168 hours of injection. Blood testosterone concentration remained in the normal range with lower variation when compared to intramuscular dosing. A single dose of 200 mg IM TE achieved supra-physiologic levels, 1658 ng/dL, 6 weeks after treatment compared to 895 ng/dL of weekly 100 mg subcutaneous TE. They also showed significantly less rise of serum estradiol and DHT levels with 50mg SC dose compared to 200mg IM injection, but similar profile with 100mg subcutaneous dose. This study demonstrates that weekly, subcutaneously administered testosterone injections lead to lower fluctuations in blood T levels.

In a retrospective cohort study, Spratt *et al.* assessed if subcutaneous testosterone would be a safe and effective alternative to IM testosterone injections (25). Patients receiving testosterone for gender transition were administered 50 mg of testosterone cypionate or enanthate weekly. All patients of the study achieved serum testosterone levels in the normal range for males. The patients also preferred subcutaneous testosterone over the IM route. These results showed that subcutaneous testosterone was safe and effective, and preferred over IM testosterone.

2.3. Complications associated with common treatment approaches

Using testosterone alone has been associated with a number of undesirable effects such as infertility, testicular atrophy, gynecomastia, and osteoporosis (28, 29). Testosterone in combination with other hormones and non-testosterone hormones and factors have been employed or studied. An approach to treating low testosterone is treatment with human chorionic gonadotropin (hCG) and an aromatase inhibitor (30, 31). The role of hCG in treatment of low testosterone is its mimicking of luteinizing hormone (LH). This leads to the stimulation of the Leydig cells of the testes to produce testosterone.

Treatment with hCG alone has been used in men with hypogonadism for enhanced spermatogenesis and testicular volume enhancement (30-32). Hsieh *et al.* found that low dose hCG co-administration helped to maintain normal semen parameters in hypogonadal men, allowing the maintenance of fertility in these men while on testosterone-replacement therapy (33). Burris *et al.* demonstrated that treatment with hCG can enhance spermiogenesis in men with isolated hypogonadotropic hypogonadism (34). Given these findings, there is a possibility that the addition of hCG to testosterone treatment regimens can mitigate the effects that occur with testosterone-only therapy.

Another effect of testosterone-only treatment is the production of higher levels of estrogen due to the conversion of testosterone to estrogen by aromatase enzyme. This has been addressed by combining testosterone treatment with administration of an aromatase inhibitor (35-38). Another treatment approach under investigation is the combination of oral testosterone with 5α-reductase inhibitor such as dutasteride. Amory *et al.* found that this approach increases the bioavailability of testosterone in men with hypogonadism (39). Wada *et al.* observed that oral testosterone and dutasteride provides positive effects on bone without causing prostate growth (40).

We postulate that shorter testosterone injection intervals using lower doses can further lessen the cyclical peaks and lows leading to more stable blood testosterone levels, a pattern resembling normal physiologic hormone activity. The application of daily subcutaneous testosterone (DST) injections is rarely used by most clinics. DST therapy combines the benefits of achieving stable blood concentrations seen with topical or pellet routes of administration, but avoids many of the adverse effects such as the serious risk of transference or invasiveness, respectively. Further, subcutaneous injections are easier to self-administer than intramuscular injections and associated with significantly less discomfort. The cost of in-center administration is also mitigated by this method, which may lead to overall savings in healthcare expenditure.

A treatment for TD that takes advantage of the pharmacokinetic benefits of daily testosterone administration with limited adverse effects would have a significantly positive impact on the therapeutic options for low testosterone. Also, co-administration with hCG and aromatase inhibitor may add to the clinical benefits of testosterone therapy and further prevent undesirable clinical outcomes associated with testosterone-only therapy. To determine the effectiveness of combining the DST method, hCG, and aromatase inhibitor, we conducted a retrospective analysis of men diagnosed or treated for low testosterone at our Seaside Medical Practice from 2009-2016. We determined the changes in various hormones and factors including serum total testosterone, free testosterone, % free testosterone, estradiol, SHBG, LH, FSH, DHT, DHEA-S, PSA, pregnenolone, and hemoglobin.

3. METHODS

A retrospective analysis of men diagnosed with and treated for TD was conducted. Review was performed on 356 male patients identified on the medical practice electronic medical database. Fifty-four of these met the following criteria of inclusion in the study: any age (resulting age range of those included in the study is 26-82 years), any race, have at least 2 laboratory points of total testosterone level, and prescribed daily subcutaneous testosterone injection per DST Method (Daily Subcutaneous Testosterone Method). The study was approved by the Seaside Medical Practice Institutional Review Board. Informed consent was not required.

Table 1. Laboratory values for studied hormones/factors

Measured hormones/factors Normal Lower Limit Laboratory Values for Men	Normal Lower Limit Laboratory Values for Men	Lab Date 1		Lab Date 2	
		Mean	SEM	Mean	SEM
Total T (ng/dL)	346 ng/dL	385.17	21.75	846.78	45.35
Free Serum T (pg/mL)	72 pg/mL	40.13	7.20	118.88	19.18
Free Direct (ng/L)	7 ng/L	30.60	7.0	106.34	35.37
% free T	1.6	1.34	0.26	1.50	0.10
DHT (pg/mL)	112 pg/mL	42.75	6.58	56.67	7.56
SHBG (nmol/L)	35 nmol/L	35.44	3.78	33.48	3.73
DHEA-S (µg/dL)	200 (µg/dL)	217.15	20.14	333.46	23.85
PSA (ng/mL)	<2 ng/mL (under 40 yrs) <5.3 ng/mL (under 80 yrs) <7.2 ng/mL (≥ 80 yrs)	1.13	0.50	0.99	0.15
Pregnenolone (ng/dL)	33 ng/dL	35.92	4.73	35.86	5.85
Estradiol (pg/mL)	35 pg/mL	24.99	1.65	25.97	2.65
LH (mIU/L)	1.0 mIU/L	5.43	0.70	1.16	0.63
FSH (mIU/L)	1.0 mIU/L	4.74	0.59	1.60	0.74
Hemoglobin (g/dL)	14 g/dL	15.20	0.16	15.37	0.42

The testosterone was administered as testosterone cypionate (7-18 mg) in more than 95% of the patients included in the study; the remaining received a combination of testosterone cypionate and testosterone enanthate. The testosterone was administered via subcutaneous injection using a 30 gauge, 1/2-inch hypodermic needle into the outer thigh, biceps, or gluteal muscles. The daily injection dose levels mirror the gonads' release of testosterone in small pulses throughout the day equaling about 4-9 mg per day (41-44). The patients also self-administered hCG (220 units two days per week, Mondays and Fridays) via subcutaneous injection with a 30 gauge, 5/16-inch hypodermic needle into the mid-abdominal wall. Anastrozole was administered as a once daily oral 0.25-0.5. mg tablet. This approach involved administering smaller subcutaneous daily injections versus large intramuscular weekly or bi-weekly injections.

3.1. Statistical analysis

The study endpoints were the change between two measurement dates (before and after start of testosterone treatment) in the levels of the hormones and factors studied (total testosterone, free testosterone, % free testosterone, estradiol, SHBG, LH, FSH, DHT, DHEA-S, PSA, pregnenolone, and hemoglobin). Differences in the studied hormones and factors between the two laboratory testing dates were analyzed using paired t-test. Statistical significance was determined with a p value less than 0.05. The time range between testing dates was 3 to13 months with an average of 6.7 months. Since testosterone levels vary based on age range, analyses (paired t-tests) were also conducted for the two primary measures for testosterone deficiency (total testosterone and free serum testosterone) for three different age groups (20-39, 40-59, and 60+). Although ethnicity and race can affect differences in testosterone levels, this factor could not be analyzed due the nature of a retrospective chart review regarding available patient populations available. The practice facility's patient population at the time of the study was predominately of the white race.

4. RESULTS AND DISCUSSION

Table 1 shows the means and standard error of the means (SEMs) for the measured blood values between the pre- and post-testosterone (T) treatment groups (lab dates 1 and 2). Total T increased from 385.17± 21.75 ng/dL to 846.78 ± 45.35 ng/dL ($p<0.01$), free serum T from 40.12 ± 7.20 pg/mL to 118.88 ± 19.18 pg/mL ($p<0.01$), and direct free T increased from 30.60 ± 7.90 pg/mL to 106.34 ± 35.37 pg/mL ($p<0.05$). There was also a significant increase of DHEA-S after treatment from 217.15 ± 20.14 µg/dL to 333.46 ± 23.85 µg/dL ($p<0.01$). LH and FSH both decreased (5.43 ± 0.70 IU/L to 1.16 ± 0.63 IU/L ($p<0.01$) and 4.74 ± 0.59 mIU/L to 1.60 ± 0.74 mIU/L ($p<0.05$), respectively). No significant differences in the % free T and the levels of estradiol, SHBG, DHT, PSA, pregnenolone, or hemoglobin were observed.

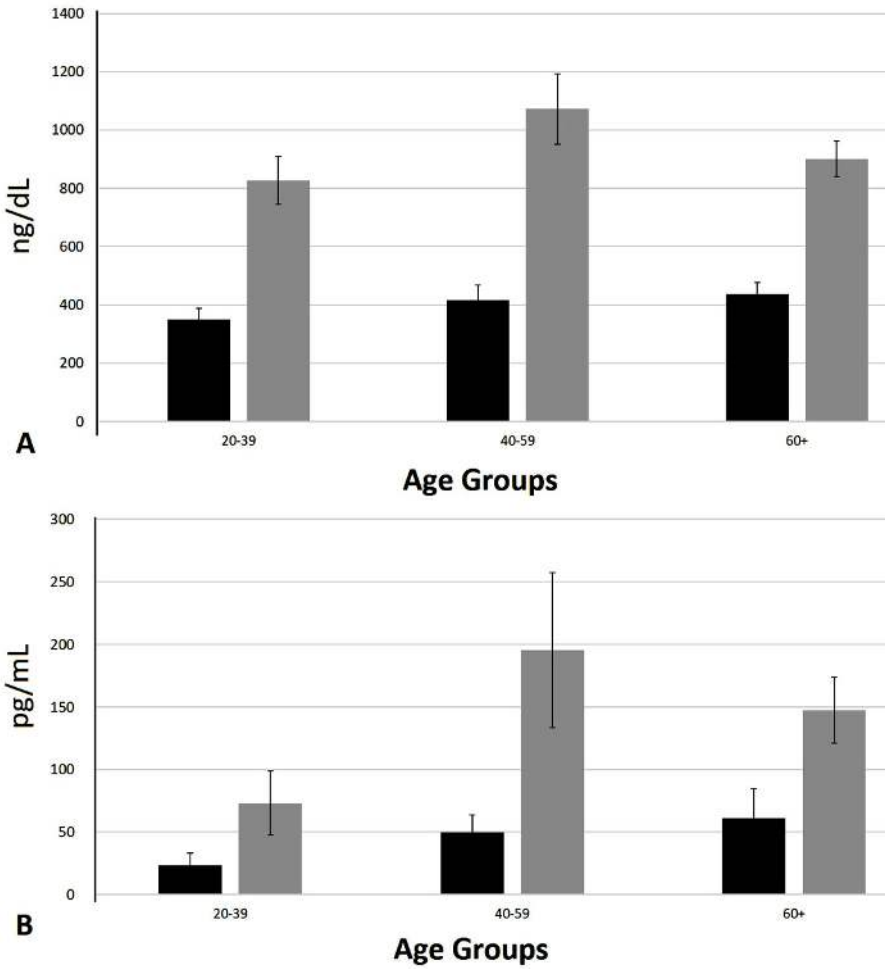

Figure 1. A. Plotted are the mean ± SEM for total testosterone (T) for three different age groups (20-39, 40-59, and 60+). Total T (ng/dL) was measured by liquid chromatography with mass spectroscopy. B. Plotted are the mean ± SEM for serum free testosterone for the same three different age groups. Free serum T (pg/mL) was measured using the equilibrium ultrafiltration assay. Dark gray bars refer to lab date 1, and light gray bars refer to lab date 2.

Figure 1A shows the differences in total testosterone measurements between the two laboratory testing dates by age group,and figure 1B shows the differences in serum free testosterone measurements. In the 20 to 39-year age group, total testosterone increased from 349.50 ± 38.39 ng/dL to 827.00 ± 81.92 ng/dL ($p<0.01$) and free serum testosterone increased from 23.32 ± 10.05 to 73.04 ± 25.57 pg/mL ($p<0.05$). In the 40 to 59-year age group, total testosterone increased from 416.11 ± 52.04 ng/dL to 1072.00 ± 120.33 ng/dL ($p<0.01$) and free serum testosterone increased from 49.72 ± 14.15 to 195.26 ± 61.81 pg/mL ($p<0.05$). Finally, in the 60 and over year age group, total testosterone increased from 436.80 ± 49.31 ng/dL to 901.13 ± 60.47 ng/dL ($p<0.01$) and free serum testosterone increased from 60.84 ± 23.89 to 147.22 ± 26.40 pg/mL ($p<0.05$). Therefore, the treatment caused increases in testosterone regardless of age group.

The results show that treatment with testosterone, hCG, and anastrozole increased testosterone levels in men with low or low normal testosterone. Although the pre-treatment mean serum testosterone of our study is 385 ng/L, free serum testosterone is below normal limits (346 ng/dL) as defined by Wang et al (13). As previously mentioned, it is not recommended to rely solely on clinical laboratory values for the diagnosis of low testosterone. The men of our study all had clinical signs and symptoms of low testosterone and free testosterone levels below normal leading to a diagnosis of TD. The DST method did not lead to supra-physiologic or highly variable testosterone levels as frequently as that reported in the literature for IM administration method (where peak testosterone concentration is high at the beginning of the injection period and very low toward end).

This treatment method involved the administration of the aromatase inhibitor anastrozole throughout testosterone-treatment, which accounted for stable estradiol levels pre and post-testosterone

therapy (40). Changes in estradiol levels are associated with increased adiposity, mood swings, decreased libido, and gynecomastia in men treated with testosterone replacement. There were no post-treatment reports by the patients of the study of adverse changes in libido. Rare reports of gynecomastia were noted. In a study by Pastuszak et al., some of the study participants on testosterone-therapy had higher estradiol levels, which was successfully treated with aromatase inhibitor (45). The findings reported by Pastuszak et al. were consistent despite variable laboratory testing intervals.

The DST therapy method used was not associated with increase in hemoglobin. Higher changes in hemoglobin and hematocrit occur more frequently with IM vs. topical or pellet administration, a trend not observed in our sample (7). Testosterone replacement inhibits hepcidin activity, thereby leading to increased iron absorption and increased erythropoiesis. It is plausible that smaller daily testosterone injections, resembling physiologic secretion, do not affect hepcidin activity to the same degree that is seen with supra-physiologic testosterone level from weekly IM injections.

The treatment was associated with decreases in LH and FSH. This is the opposite effect seen with aromatase only treatment. Leder et al. treated men aged 62 to 74 with 1 mg of anastrozole orally once or twice per week for 12 weeks (46). The treatment increased testosterone, LH, and FSH levels. It is known that LH and FSH stimulate sperm production in men. However, high FSH levels in men are associated with impaired testicular function due to advanced age or other factors (e.g., alcohol use, chemotherapy) that adversely affect testicular function. The average pre- and post-treatment FSH level was still at the lower end of the normal range, while LH was slightly below the lower limit provided in the University of Iowa Laboratory Services Handbook (47). The lowering of FSH and LH represent a negative feedback effect due to the testosterone treatment. No patients required fertility treatment or reported infertility as a result of DST therapy method. Rare reports of testicular atrophy were noted.

The increase in DHEA-S with testosterone treatment indicates that the adrenal glands responded to testosterone administration. Although the role of DHEA co-administration cannot be ruled out, a study by Brown et al. showed that a onetime ingestion of up to 50 mg of DHEA or chronic daily oral dosing with as much as 150 mg/day does not affect serum testosterone and estrogen concentrations. In this study, the DHEA-S levels rose to an average value considered normal for males under age 40 (48, 49). The active DHEA has weak androgenic activity and is converted to testosterone in men. Therefore, the increase in DHEA-S may further help raise testosterone levels in men on testosterone therapy.

Changes in SHBG were not observed, and values before and after testosterone treatment remained in the normal range. SHBG binds and transports the sex hormones. Anastrozole treatment can cause a decrease in SHBG levels (38). Low SHBG levels have been associated with an increased risk of developing Type 2 diabetes (5). Estrogenic compounds in men can increase SHBG levels leading to signs of hypogonadism. The anastrozole treatment did not lead to an increase in estradiol or SHBG levels in this study.

In men, approximately 5% of testosterone is reduced to DHT by 5α-reductase. Although DHT is not particularly elevated in benign prostatic hyperplasia (BPH), it has a role in both BPH and male pattern baldness (50, 51). There was an increase in the average DHT level, but this change was not statistically significant. Gore et al. indicate that testosterone therapy can increase PSA and exacerbate prostate cancer (52). However, Debruyne et al. conducted a study using the Registry of Hypogonadism in Men (RHYME) to determine the effects of testosterone-replacement therapy on indicators of prostate health in hypogonadal men (53). The results of the analysis suggested that testosterone-replacement therapy was not associated with elevated PSA levels or a higher risk of prostate cancer. Our results show that the studied testosterone-treatment method did not lead to an increase in PSA levels. Pregnenolone is synthesized from cholesterol and is a precursor for various hormones including testosterone. Pregnenolone itself has functional roles and may be associated with improvement in depressive disorders (54). The testosterone therapy used by our facility was not associated with changes in blood pregnenolone levels.

Although there were statistically significant increases in testosterone levels using the DST therapy method with anastrozole and hCG co-treatment, there were some limitations of the study. There was variability between individuals in the time interval between the 2 laboratory testing dates. Also, a few of the patients received two forms of testosterone for therapy. Additional factors that could influence the observed changes in blood testosterone levels were supplements and other treatments taken by the patients before, during, and/or after the initiation of testosterone therapy. Some patients that received the DST treatment also took pregnenolone, HGH, DHEA, or vitamin D. These treatments may have affected the magnitude of observed testosterone levels after the testosterone therapy. Finally, the ages of the individuals varied greatly. However, an analysis based on age group showed consistent increases in testosterone levels. However, the magnitude of responses to testosterone treatment may have differed according to the age and health status of the individuals treated.

5. DISCUSSION

Despite the noted limitations, the consistent and statistically significant increases in the three testosterone values suggest that the DST therapy method used for the patients included in the study has positive effects on testosterone level status. Co-treatment with an aromatase inhibitor and hCG appear to have prevented some of the adverse effects commonly observed with testosterone-only treatment. These findings can serve as a basis for further studies (including prospective controlled trials) to determine the range of benefits and better define the expected outcomes with the DST therapy method using aromatase inhibitor and hCG co-treatment.

6. CONCLUSIONS

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