INTERSEX AND GENDER IDENTITY DISORDERS

Physical and Psychological Effects of Gender-Affirming Hormonal Treatment Using Intramuscular Testosterone Enanthate in Japanese Transgender Men

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ABSTRACT

Introduction: The evidence on gender-affirming hormonal treatment (HT) for transgender persons is still insufficient.

Aim: To characterize the physical and psychological effects of HT using testosterone enanthate in transgender men, and to validate the safety of this treatment.

Methods: A total of 85 Japanese transgender men who were followed up for at least 1 year at our gender clinic from 2004 to 2017 were included in this study. All self-reported effects that they recognized and regularly acquired laboratory data were investigated after initiation of HT.

Main Outcome Measure: HT mainly using testosterone enanthate 250 mg every 2 weeks caused the most desired physical effects to appear promptly and effectively, whereas small but not negligible numbers of undesired physical and psychological effects were also confirmed.

Results: The initial dose of testosterone enanthate was 250 mg for 72 (84.7%) subjects, and the injection interval was maintained every 2 weeks for 70 (82.3%). Most physical effects appeared within 6 months. A deepened voice (87.1%), cessation of menses (78.8%), acne (69.4%), and facial (52.9%)/body (37.6%) hair growth occurred within 3 months. Although recognition of psychological effects was rare, emotional instability (9.4%) and increased libido (7.1%) appeared in the relatively early phase after beginning HT. The mean values for red blood cells, hemoglobin, uric acid, and alkaline phosphatase were significantly increased for 2 year. During the observation period, there were no life-threatening adverse effects in any subjects.

Conclusion: The present HT strategy is effective and safe for Japanese transgender men. The information from self-reported effects and objective data from blood tests can help both physicians and transgender men to understand testosterone HT. **Kirisawa T, Ichihara K, Sakai Y, et al. Physical and Psychological Effects of Gender-Affirming Hormonal Treatment Using Intramuscular Testosterone Enanthate in Japanese Transgender Men. Sex Med 2021;9:100306.**

Key Words: Gender Dysphoria; Gender Incongruence; Hormonal Treatment; Testosterone; Transgender Men

INTRODUCTION

The terminologies transgender and gender incongruence are applied for persons with gender identities that are not aligned with the sex assigned at birth.[1] If gender incongruence persists and leads to distress, there can be gender dysphoria.[2] Although the basis of treatment for transgender persons is mental support, if discomfort persists for a certain period, physical treatments (gender-affirming treatment) to align appearance with gender identity would be recommended. One of them is hormonal treatment (HT).

HT using exogenous testosterone is administered to transgender men.[3] Although the recommended treatment methods and general masculinizing effects of testosterone HT are referred to in guidelines,[3] many other side effects are also suggested.[4] Furthermore, psychological effects have been noted.[5] On the

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other hand, the data for Japanese transgender men have not been elucidated. In Japan, medical expenses of HT for transgender persons are not covered by health care insurance at present, and there is no guideline for proper HT enforcement. Therefore, verification of the effects and safety of HT has been insufficient, and accumulation of evidence about HT is important.

In this study, we set testable hypothesis that all kinds of effects of testosterone HT for Japanese transgender men would be appeared at the same time and frequency as previously reported, and the safety be also guaranteed. We focused on the details of testosterone HT and aimed to clarify the subjective and objective effects of HT via physical and psychological profiles. We intended to determine what kinds of effects occurred and when these effects were recognized by the patients themselves after beginning HT.

MATERIALS AND METHODS

This was a retrospective observational study. The study protocol was approved by the institutional review board of the study site (approval no. 302-200) and was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Because this study is a retrospective one, informed consent for study participants was approved for exemption by publishing its contents on website. A total of 194 transgender men visited our gender clinic from October 2004 through March 2017. Of them, 85 transgender men who started HT and were followed up for at least 1 year as outpatients were included in this study. Exclusion criteria were the absence of HT information, having already started HT, having started HT with an oral agent, or not having started HT despite having approval for treatment.

Before transgender persons commence HT in our clinic, a medical checkup including blood cell counts and biochemical examinations, hormonal tests, and cancer screening is usually required. In general, intramuscular injection of 250 mg of testosterone enanthate every 2 weeks was selected as the initial HT regimen. Thereafter, the dose and interval were adjusted depending on physical condition. It was routine for physicians to query the subjects at every time they visited for injection about the onset of physical or psychological changes after starting HT. In addition, routine blood tests were performed at 3, 6, and 12 months. If the subjects reported any adverse reactions associated with HT or had abnormal laboratory data during HT, the dose and injection interval of testosterone could be flexibly modified. After 1 year, a self-report on HT effects was done only when the subjects felt any changes they were concerned about, and blood testing was performed every 1 year.

We obtained the following data from clinical records. For patient characteristics, we investigated age at the beginning of HT and the details of HT (the initial dosage, dose interval, and whether the dosage was changed during treatment). For the effects of HT, we extracted all self-reported physical and psychological events and the time points when they were recognized. In addition, the results of routine blood tests were evaluated. If the subjects underwent gender-affirming surgery (including oophorectomy) within 2 years, we defined the day of surgery as the end of the follow-up period because non-negligible bodily changes had occurred.

Statistical Analyses

For descriptive statistics, the data were expressed as the mean ± standard deviation (SD) and/or median [range]. For the evaluations for sequential data in blood tests, if there were missing values, all cases for which data could be obtained at each point were used for analysis. Analysis of variance followed by the Dunnett test was used for statistical comparisons between groups. A P-value of <0.05 was considered statistically significant. All statistical analyses were performed with EZR statistical software (Saitama Medical Center, Jichi Medical University),[6] which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R Commander (version 1.6-3) that includes the statistical functions that are frequently used in biostatistics.

RESULTS

Study Population and Hormonal Treatment Regimen

The characteristics of the 85 transgender men are shown in Table 1. The mean age at initiation of HT was 27.1 ± 6.5 years, and a total of 12 subjects had already undergone mastectomy. The initial dose of testosterone enanthate was 250 mg for 72 subjects (84.7%) and 125 mg for 13 (15.3%). During the observation period, the dose was changed for 5 subjects (from 250 mg to 125 mg for 3 and from 125 mg to 250 mg for 2). The reasons for this were complaints such as fatigue, scalp hair loss,

Table 1. Characteristics of 85 transgender men

Age at the start of HT (years), mean ± SD	27.1 ± 6.5
Details of testosterone enanthate dose during HT, number (%)	
250 mg	69 (81.1%)
125 mg	11 (13.0%)
250 mg → 125 mg	3 (3.5%)
125 mg → 250 mg	2 (2.4%)
Injection interval (weeks), median [range]	2 [2 - 4]
Undergone mastectomy	
At the time of HT start, number (%)	12 (14.1%)
After receiving HT, number (%)	41 (48.2%)
Undergone gender-affirming surgery, number (%)	39 (44.7%)
Time from the start of HT (months), mean ± SD	39.4 ± 24.2
Within 2 years from the start of HT, number (%)	12 (14.1)

HT = hormonal treatment; SD = standard deviation.

and emotional instability. For 70 (82.3%) transgender men, the testosterone injection interval was maintained at every 2 weeks (250 mg; 61 subjects, 125 mg; 9 subjects), but for the others it was not (every 2 to 4 weeks). Finally, 39 transgender men underwent gender-affirming surgery (12 within 24 months).

Physical and Psychological Effects of Hormone Treatment

The subjective physical and psychological effects after HT initiation are shown in Table 2. Because most symptoms were confirmed within 2 years, the observation period for each subject was 2 years in this study. The most frequently reported physical effect was a deepened voice, which was found in 74 (87.1%) transgender men, followed by cessation of menses (78.8%), acne (69.4%), facial hair growth (52.9%), and body hair growth (37.6%). These changes were noticed by subjects as early as about 1 month after the start of HT and occurred within 15 months for all subjects who were aware of the effects. The median time of onset was 1 month for deepened voice, 2 months for cessation of menses and acne, and 3 months for hair growth. Next, the effects reported in more than 10% of subjects were weight gain, clitoral enlargement, muscle gain/fat redistribution, fatigue, and vasomotor symptoms. Less commonly, effects reported by more than 5 subjects were sleepiness, vertigo, and scalp hair loss.

There were few reports of psychological symptoms, but their number was not negligible. In any case, emotional anxiety, increased libido, and increased appetite were reported within 6 months after the start of HT. Only 2 (2.4%) subjects reported increased aggression in the early phase of HT.

Blood Tests

The data that showed significant changes over time are presented in Figures 1 and 2. In the blood cell counts, the number of red blood cells and the density of hemoglobin continued increasing consistently from the beginning of HT start for 2 year (Figure 1, A and B). In the biochemical test, significant changes were observed in the values of uric acid and alkaline phosphatase (ALP). These values sustainably increased for 2 year (Figure 2, A and B). On the other hand, the value of lactate dehydrogenase (LDH) showed a transient ascent at 3 months compared with that before HT, but thereafter slightly declined and did not show a significant difference (Figure 2C). However, the value kept consistently higher than

Table 2. Subjective effects in 85 transgender men recognized after intramuscular testosterone enanthate administration for 2 years

		Onset (month)
Effects	Number (%)	Mean ± SD, median [range]*
Physical		
Deepened voice	74 (87.1)	2.2 ± 2.5, 1 [0.5-14]
Cessation of menses	67 (78.8)	2.7 ± 2.7, 2 [0.5-15]
Acne	59 (69.4)	3.1 ± 2.7, 2 [0.5-13]
Facial hair growth	45 (52.9)	3.8 ± 3.3, 3 [1-15]
Body hair growth	32 (37.6)	3.6 ± 2.6, 3 [1-14]
Weight gain	20 (23.5)	4.9 ± 4.0, 4 [1-14]
Clitoral enlargement	20 (23.5)	6.4 ± 6.1, 5 [0.5-24]
Muscle gain/fat redistribution	13 (15.3)	5.9 ± 6.4, 3 [0.5-24]
Fatigue	11 (12.9)	6.5 ± 7.0, 5 [0.5-24]
Vasomotor symptoms	11 (12.9)	4.4 ± 6.9, 1 [0.5-24]
Sleepiness	6 (7.1)	5.3 ± 9.2, 2 [0.5-24]
Vertigo	5 (5.9)	4.7 ± 3.2, 5 [0.5-9]
Scalp hair loss	5 (5.9)	8.6 ± 9.0, 5 [2-24]
Menstrual cramps	4 (4.7)	12, 14, 24, 24
Metrorrhagia	3 (3.5)	1, 12, 24
Palpitation	2 (2.4)	0.5, 12
Shoulder stiffness	2 (2.4)	0.5, 3
Edema	2 (2.4)	1, 5
Breast pain	1 (1.2)	12
Psychological		
Emotional instability	8 (9.4)	3.9 ± 4.8, 2.5 [0.5-14]
Increased libido	6 (7.1)	2.9 ± 4.0, 1.5 [0.5-11]
Increased appetite	5 (5.9)	4.2 ± 4.8, 3 [0.5-12]
Increased aggression	2 (2.4)	1, 1

SD = standard deviation.
*If the numbers were less than 5, each of the data was presented.

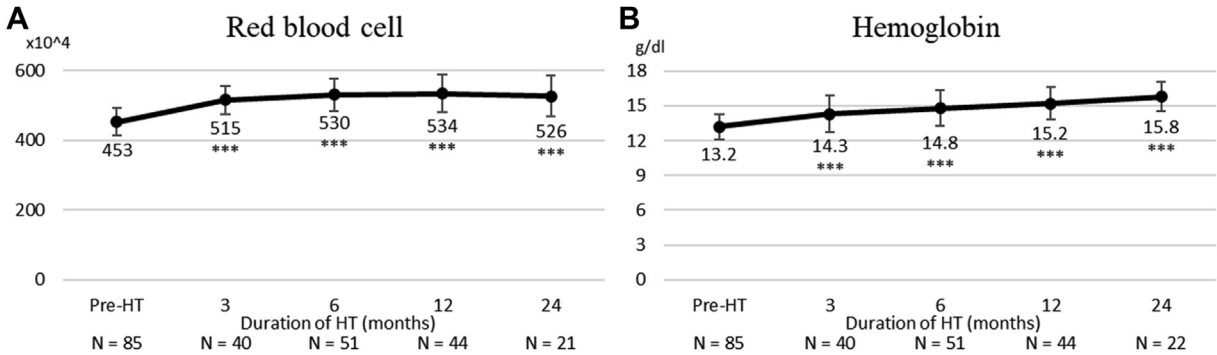

Figure 1. The changes of blood cell count during HT. (A) red blood cell and (B) hemoglobin. Each value and error bar represent mean value and standard deviation. ***$P < .001$: significant differences from pre-HT (ANOVA followed by Dunnett test). HT = hormonal treatment; Pre-HT = the time before HT started; N = the number of cases for which data could obtain at each evaluation point.

that before HT. Significant changes were not observed over time for other parameters such as total bilirubin, cholesterol, triglycerides, transaminase, and coagulation parameters (D-dimer) (data not shown). There were no life-threatening adverse effects in any of the transgender men during the observation period, and HT was safely conducted.

DISCUSSION

The goals of HT are to suppress endogenous sex hormone secretion determined by the person's genetic/gonadal sex and to maintain sex hormone levels within the normal range for the person's affirmed gender.[3] For transgender men, an androgen is given to induce masculinization, and "desired side effects" of the androgen. Although, it is desirable that these side effects appear early and effectively, there is no guideline for proper use in Japan. In the clinical practice guidelines of the endocrine society,[3] recommended HT regimens are described. In the case of testosterone enanthate, it is indicated that this preparation should be injected at a dose of 100 mg to 200 mg every 2 weeks. Then the serum total testosterone level should be measured every 3 months until the values are in the normal physiological male

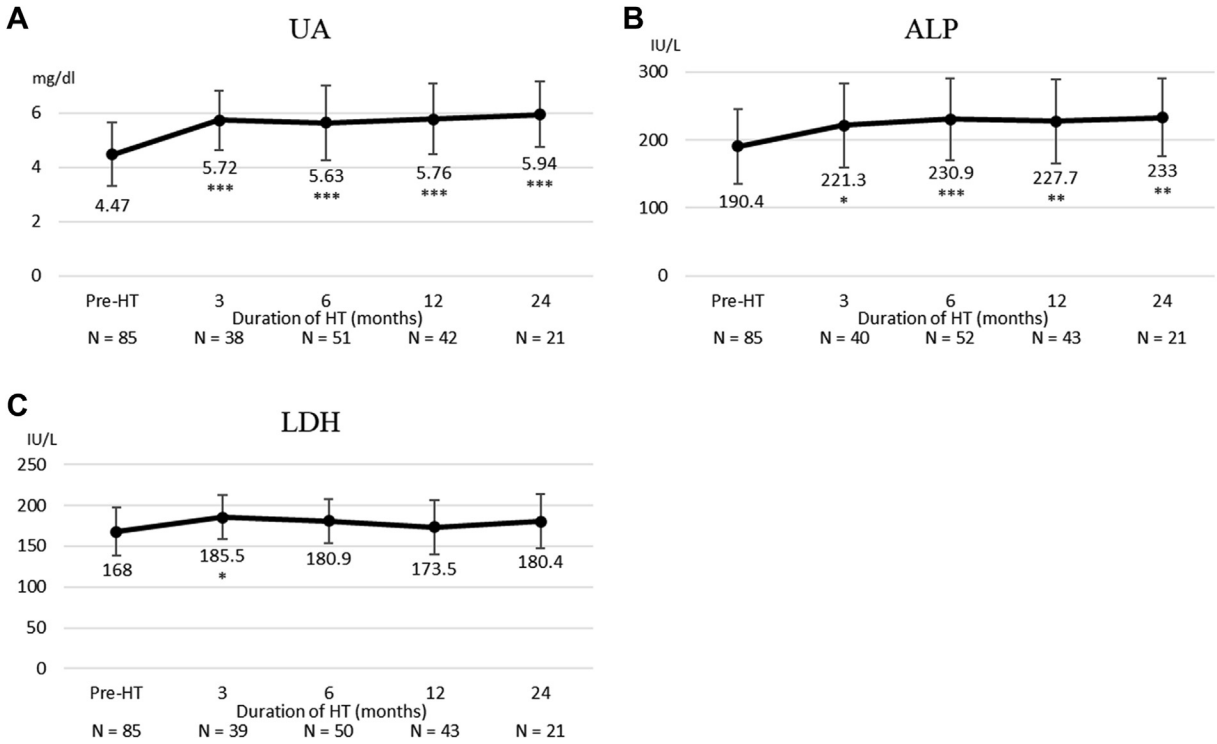

Figure 2. The changes of biochemistry data during HT. (A) UA, (B) ALP, (C) LDH. Each value and error bar represent mean value and standard deviation. *$P < .05$, **$P < .01$, ***$P < .001$: significant differences from pre-HT (ANOVA followed by Dunnett test). UA = uric acid; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; HT = hormonal treatment; Pre-HT = the time before HT started; N = the number of cases for which data could obtain at each evaluation point.

range. Moreover, the testosterone level should be measured at intermediate times between injections and adjusted as per the results.[3]

On the other hand, in our country, there is no ideal HT regimen with high-quality evidence, including a recommended follow-up method. Thus, the physical and psychological effects and safety of HT have not been fully evaluated. Therefore, in this study, we aimed to clarify what kinds of effects of HT using testosterone enanthate occurred, and whether its safety could be guaranteed. We found that HT mainly using 250 mg of testosterone enanthate every 2 weeks induced most "desired" physical effects reported previously promptly and effectively. Although some blood test results significantly changed, the final result was acceptable. On the other hand, the incidences of several physical and psychological effects were low but they seemed to be "nondesired."

The proper testosterone dose for transgender men is controversial. Nakamura et al reported that the onset of physical changes was more prevalent in a group receiving a higher dose, and there were dose-dependent effects observed among treatment groups.[7] However, after 6 months, there were no dose-dependent effects regarding the percentage of subjects with therapeutic effects. Thus, the authors recommended testosterone administration of 125 mg every 2 weeks.[7] In the present study, we selected 250 mg every 2 weeks in principle, and most physical effects appeared within 3 months. In accordance with our recent report that evaluated the pharmacokinetics of a single injection of 250 mg of testosterone enanthate, it is appropriate to administer it every 2 weeks to maintain the recommended serum testosterone level.[8] In addition, the *in vivo* distribution and clearance of testosterone after intramuscular administration greatly varied due to interindividual variations in its bioavailability.[8] Therefore, if we use 250 mg of testosterone enanthate as the initial HT, the masculinization effects would be visible more quickly than with 100 mg or 200 mg. By contrast, after the desired virilization has been obtained, maintenance administration at 100 mg or 200 mg may be appropriate in consideration of safety.

In this study, relatively rare but non-negligible psychological effects such as emotional instability or increased libido/appetite/aggression were also confirmed. Among them, emotional instability (including anxiety) is one of the psychological effects several studies previously reported. In accordance with these reports, transgender persons already have psychiatric disorders before HT, which may influence their mental stability after HT.[5] In addition, social support, quality of life, and sexual orientation were also suggested to influence their mental stability.[9] On the other hand, some reports proposed the possibility of positive effects on mental stability by conducting HT.[10–12] Our present study did not confirm whether the subjects were affected by psychiatric disorders; thus further studies should be conducted to elucidate the mechanisms of these effects.

Blood test results confirmed significant changes in the values of red blood cells, hemoglobin, uric acid, and ALP between the point before HT and all other periods. These results were concordant with data previously reported except for ALP.[7,13] Regarding it, we speculate that elevation of the ALP value may reflect an increase of bone mineral density, which is suggested to be related to testosterone administration.[14] Unfortunately, we did not evaluate bone density, so the correlation between ALP and bone density was not confirmed. On the other hand, the present study confirmed a transient increase in the LDH value. LDH is distributed in various organs such as the liver and muscles. Therefore, the increase might be attributable to damage to these organs by testosterone. However, in the case of the liver, it is known that oral testosterone administration causes hepatotoxic effects, but intramuscular injection is nonhepatotoxic because it circumvents the first-pass hepatic metabolism.[4,15] Meanwhile, in a previously reported animal experiment, testosterone enanthate injection combining load training resulted in significant elevation of serum LDH.[16] That is, elevation of LDH implies the possibility that HT promotes muscle gain followed by transient increased physical activity, although the exact reason is not clear.

Finally, the limitations of this study should be addressed. First, this study was retrospective, so we may not have extracted all the effects that the participants recognized. In addition, we could not evaluate the degrees of these effects. When one effect occurred at one point in time, it might have disappeared at other points. Second, there were no strict criteria about the dose and interval of testosterone, as these were determined by each physician. Third, the reported psychological effects were the subjective emotions of each individual and thus might lack objectivity. Therefore, a prospective study evaluating the chronological changes of each effect using validated questionnaires is needed in the future. However, the present study clarified subjective and objective physical and psychological effects due to testosterone HT as far as possible in a relatively large number of Japanese transgender men.

CONCLUSION

We retrospectively investigated the influences of gender-affirming hormone treatment using intramuscular testosterone enanthate in Japanese transgender men. Most of the results were similar to those reported previously. However, small but not negligible numbers of nondesired physical and psychological effects considered to be due to HT were also confirmed.

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STATEMENT OF AUTHORSHIP

Takahiro Kirisawa, Acquisition of Data, Analysis and Interpretation of Data, Drafting the Article, Final Approval of the

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REFERENCES

1. Safer JD, Tangpricha V. Care of transgender persons. **N Engl J Med 2019;381:2451-2460.**
2. Diagnostic and statistical manual of mental disorders. Fifth edition. Washington, DC: American psychiatric publishing; 2013.
3. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocline treatment of gender-Dysphoric/gender-Incongruent persons: an Endocline society clinical practice guideline. **J Clin Endocrinol Metab 2017;102:3869-3903.**
4. Rhoden EL, Morgentaler A. Risks of testosterone-replacement therapy and recommendations for monitoring. **N Engl J Med 2004;350:482-492.**
5. Motta G, Crespi C, Mineccia V, et al. Does testosterone treatment increase Anger expression in a Population of transgender men? **J Sex Med 2018;15:94-101.**
6. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. **Bone Marrow Transpl 2013;48:452-458.**
7. Nakamura A, Watanabe M, Sugimoto M, et al. Dose-response analysis of testosterone replacement therapy in patients with female to male gender identity disorder. **Endocr J 2013; 60:275-281.**
8. Ichihara K, Masumori N, Fujii S, et al. Pharmacokinetics of testosterone enanthate after intramuscular injection for transgender men. **Androgens: Clin Res Ther 2020;1:1.**
9. Meier SC, Pardo ST, Labuski C, et al. Measures of clinical health among female-to-male transgender persons as a function of sexual orientation. **Arch Sex Behav 2013;42:463-474.**
10. Ruppin U, Pfäfflin F. Long-term follow-up of Adults with gender identity disorder. **Arch Sex Behav 2015;44:1321-1329.**
11. Colizzi M, Costa R, Todarello O. Transsexual patients' psychiatric comorbidity and positive effect of cross-sex hormonal treatment on mental health: results from a longitudinal study. **Psychoneuroendocrinology 2014;39:65-73.**
12. Heylens G, Verroken C, De Cock S, et al. Effects of different steps in gender reassignment therapy on psychopathology: a prospective study of persons with a gender identity disorder. **J Sex Med 2014;11:119-126.**
13. Kurahashi H, Watanabe M, Sugimoto M, et al. Testosterone replacement elevates the serum uric acid levels in patients with female to male gender identity disorder. **Endocr J 2013; 60:1321-1327.**
14. Snyder PJ, Peachey H, Hannoush P, et al. Effect of testosterone treatment on bone mineral density in men over 65 years of age. **J Clin Endocrionol Metab 1999;84:1966-1972.**
15. Bagatell CJ, Bremner WJ. Androgens in men – uses and abuses. **N Engl J Med 1996;334:707-714.**
16. Karbasi S, Zaeemi M, Mohri M, et al. Effects of testosterone enanthate and resistance training on myocardium in Wister rats; clinical and anatomical pathology. **Andrologia 2018;50.**