# ORIGINAL

# Dose-response analysis of testosterone replacement therapy in patients with female to male gender identity disorder

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**Abstract.** Gender identity disorder (GID) is a conflict between a person's actual physical gender and the one they identify him or herself with. Testosterone is the key agent in the medical treatment of female to male GID patients. We conducted a dose-response analysis of testosterone replacement therapy (TRT) in 138 patients to determine the onset of the therapeutic effects. The TRT consisted of intramuscular injection of testosterone enanthate and patients were divided into three groups; 250 mg every two weeks, 250 mg every three weeks and 125 mg every two weeks. The onset of deepening of voice, increase in facial hair and cessation of menses was evaluated in each group. At one month after the start of TRT, the onset of these physical changes was more prevalent in the group receiving the higher dose of testosterone, and there were dose-dependent effects observed between the three treatment groups. On the other hand, at six months after the start of TRT, most of the patients had achieved treatment responses and there were no dose-dependent effects with regard to the percentage of patients with therapeutic effects. No significant side effects were observed in any of the treatment groups. We demonstrated that the early onset of the treatment effects of TRT is dose-dependent, but within six months of starting TRT, all three doses were highly effective. Current study provides useful information to determine the initial dose of TRT and to suggest possible changes that should be made in the continuous dosage for long term TRT.

Key words: Gender identity disorder, Testosterone, Female to male, Dose response analysis, Estradiol

**GENDER IDENTITY DISORDER** (GID) is a condition where an individual experiences significant gender dysphoria or discontent due to the divergence between their biological sex and their actually perceived sex. The concept of GID was introduced in the 20<sup>th</sup> century [1], and the affected patients usually indicate an apparently normal somatic sexual differentiation and hope to live as the member of the opposite gender to their innate one. The number of patients diagnosed with the condition has been increasing in the past decade. Currently, a broad approach toward to treatment of GID is conducted from various aspects of law, sociology, psychol-

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ogy and medicine to effectively manage the disorder.

The aim of the treatment for GID patients is to provide relief from the dichotomy between body habitus and sex identity by the induction of desired mental changes and maintenance of an acceptable physical state of the opposite sex. With regard to the history of the medical approach towards GID, surgical intervention was first conducted in 1907, and hormone therapy using testosterone replacement in female to male GID was started from 1935 after the discovery of testosterone [2]. Currently, the treatment for GID consists of psychotherapy, hormone replacement therapy and sex reassignment surgery. The therapeutic strategy is often determined, and therapies are started, by incorporating the patient's preferences or based on their self-determination.

Testosterone is the key agent used in the medical treatment of female to male GID patients. Testosterone replacement therapy (TRT) is often performed for the

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female to male GID patients to transform the female hormonal profile into the male one by administering exogenous androgen. The goal of TRT treatment is to induce virilization, including a male pattern of hair, a male voice and physical contours, and stopping menses [3, 4]. However, the adverse effects of TRT are real and apparent. Mortality and morbidity associated with some treatment regimens have been reported, and the side effects include water and sodium retention, hypertension, increased erythropoiesis, increased lowdensity lipoprotein (LDL), elevation of liver enzymes, induction of obesity and acne, and emotional and psychiatric problems, such as increased aggressiveness [3-6].

The dosage used during TRT for female to male GID varies from patient to patient, because the therapeutic dose should be selected based on not only the expected effects and side effects, but also the patient's preference and physical, social and economical contexts. In recent years, TRT has become more diverse, including a variety of formulations, dosages and dose intervals. The most frequently used are 100-250 mg testosterone enanthate by intramuscular administration every 2-3 weeks [3, 4]. However, within these doses, clear standardization of the initial dose and the dose interval have not been determined. In particular, there have been few studies investigating the relationship between the testosterone dosage and the duration of time necessary for the onset of therapeutic physical responses. In this study, we report our experience with three TRT doses for the treatment of Japanese patients with female to male GID. We conducted a dose-response analysis of TRT in terms of the therapeutic effects on physical changes: the deepening of the voice, increase in facial hair and cessation of menses.

## **Patients & Methods**

## The GID clinic at Okayama University Hospital

The GID clinic at Okayama University Hospital was established in 1997 and consists of four departments: psychiatry, urology, gynecology and plastic and reconstructive surgery. All patients with female to male or male to female GID undergo a standard psychiatric evaluation to exclude major functional psychiatric disorders such as schizophrenia, mood disorders and neurosis. The GID clinic provides diagnostic assessment, counseling, genetic testing, hormonal therapy, plastic surgery and coordination of social service resources [7, 8]. All aspects of this study were approved by the Ethics Committee of Okayama University Hospital.

## **Patients**

Testosterone replacement therapy (TRT) was given to a total of 206 consecutive Japanese patients with female to male GID from August 1999 to June 2008 in the Department of Urology of Okayama University. Of these patients, 138 patients who had not received any TRT previously and could be followed up for at least six months were evaluated in this study.

## Testosterone replacement therapy and follow up

Testosterone replacement therapy was given by regular intramuscular injections of testosterone enanthate in the outpatient clinic. Patients were divided into three groups based on the doses and dose intervals: 250 mg every two weeks (group A); 250 mg every three weeks (group B) and 125 mg every two weeks (group C). The dosage was selected based on the interval that the patient could visit and the costs of the treatments. The investigation at the initial visit consisted of an interview, physical examination, chromosomal study, blood tests (CBC: complete blood cell) count, biochemical tests (ALT: alanine aminotransferase, AST: aspartate aminotransferase), coagulation parameters (PT: prothrombin time, aPTT: activated partial thromboplastin time), urine analysis, hormonal tests (testosterone, estradiol) and electrocardiogram. Three and six months after the initiation of therapy, all of the tests were repeated except for the chromosomal study and electrocardiogram. The assessment of the onset of physical changes; deepening of voice, increase in facial hair and cessation of menses, was based on the medical examinations and questions. These physical changes were evaluated as the therapeutic effects which most of the female to male GID patients expect, based on our experience. Patients who discontinued treatment or required changes in the dosage during the follow-up period were excluded from this study. Patients who received sex reassignment surgery during TRT were also excluded. A retrospective study was carried out with respect to the testosterone dosage and the onset of deepening of voice, increase in facial hair and cessation of menses.

## Hormonal measurements

The serum of the patients was obtained in the afternoon at Okayama University Hospital, and the assays for serum testosterone and estradiol were performed in the laboratory of SRL Inc., Tokyo, Japan. The serum testosterone level was measured quantitatively as the total testosterone level using an electro-chemiluminescence immunoassay (ECLIA). The standard value of the assay ranged from  $131 \sim 871$  ng/dL in males and  $11 \sim 47$  ng/dL in females. The serum estradiol level was also measured using an ECLIA method. The standard value of the assay ranged from  $15 \sim 35$  pg/mL in males and  $20 \sim 550$  pg/mL in non-pregnant females before menopause.

## Statistical analysis

The data about the serum testosterone and estradiol levels are expressed as the means  $\pm$  standard deviation (S.D.). An analysis of variance (ANOVA) was performed for the statistical analysis between the groups. The differences were considered to be statistically significant at p < 0.05.

## Results

#### **Patients**

A total of 138 Japanese patients with female to male GID were treated with testosterone replacement therapy and were evaluated in this study. There were 30 patients in group A (250 mg every two weeks), 50 patients in group B (250 mg every three weeks) and 58 patients in group C (125 mg every two weeks). The average age of the 138 patients was  $26.6 \pm 5.8$  years and their average body weight was  $57.0 \pm 10.9$  Kg. There was no statistically significant difference in the patient age and body weight between the three treatment groups.

#### **Desired clinical effects**

All patients were satisfied with the therapeutic effects of TRT and no discontents with the visit interval and costs were shown. Most of the patients reported a decline in breast size at six months after initiating the treatment. The onset of deepening of voice was determined in each patient based on the inquiry at one month and six month after starting TRT. The percentage of patients is shown in terms of the onset of deepening of voice stratified by the duration of TRT [~1 month, 1~6 months and ~6 months] (Fig. 1). Deepening of the voice within one month of starting TRT was observed in 54.5% of patients in group A, 26.1% in group B and 11.1% in group C (Fig. 1). The onset of deepening of voice by the one month examination was more com-

mon in group A. The percentage of patients is also shown in terms of the onset of an increase in facial hair and cessation of menses stratified by the duration of TRT (Figs. 2 and 3, respectively). An increase in facial hair which needed to be shaved was found in 21.7% of the patients in group A, 13.2% in group B and 11.4% in group C at one month (Fig. 2). Cessation of menses was found in 62.1% of the patients in group A, 50.0% in group B and 47.3% in group C (Fig. 3). After one month of TRT, the onset of these physical changes was more prevalent in the group receiving the higher dosage of testosterone, and indicated the dose-dependency of the effects.

We next evaluated the cumulative percentage of patients with the therapeutic effects after six months of TRT. The percentage of patients with a deepening of voice, increase in facial hair and cessation of menses was 100%, 73.9% and 96.6% in group A, 97.8%, 97.4% and 85.7% in group B, and 96.3%, 72.7% and 92.7% in group C, respectively (Fig. 4). No tendency toward dose-dependent therapeutic effects was observed in terms of these cumulative percentages.

#### Hormonal parameters

The mean serum testosterone level at three and six months after the initiation of TRT was calculated in each patient. The value actually indicates the average serum testosterone levels during TRT in each dosage group included in this study. The average testosterone levels significantly increased following TRT in all three groups, compared to the pre-treatment levels (Fig. 5A). The testosterone levels were around 700 ng/ dL in all three of the treatment groups. There were no significant differences in the testosterone levels between the groups. The serum estradiol levels significantly decreased during TRT in comparison to the pretreatment levels, and there were also no significant differences between the three treatment groups in terms of the estradiol levels (Fig. 5B).

#### Adverse effects

No significant side effects were observed in any treatment group during the observation period. The doses of TRT that were used did not lead to troublesome degrees of acne or seborrhea. There was a tendency for there to be an increase in the systolic and diastolic blood pressure during the study period, however, the change was not clinically relevant. No evidence of aggression, hostility or sleep apnea was noted dur-

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Fig. 1 The onset of deepening of voice was analyzed, and the results are presented based on each dose. The onset was determined in each patient at one month and six months after TRT initiation. The percentage of patients with the onset is shown at the indicated duration.



Fig. 2 The onset of an increase in facial hair was analyzed, and the results are presented after stratification by each dosage. The onset was determined in each patient at one month and six months after the initiation of TRT. The percentage of patients with the onset of increased facial hair is shown at the indicated durations.



Fig. 3 The onset of the cessation of menses was analyzed, and data are presented after stratification for each dosage group. The onset was determined in each patient at one month and six months after the initiation of TRT. The percentage of patients is shown at the indicated durations.



Fig. 4 The cumulative percentage of patients with the therapeutic effects was analyzed at six months after the initiation of TRT.



Fig. 5 The serum levels of testosterone (A) and estradiol (B) are shown before and during TRT. The mean levels of testosterone and estradiol at three and six months after the initiation of TRT were calculated in each patient. The value after treatment indicates the average levels of testosterone and estradiol during TRT for each group. [n.s.] indicates no significant difference between the groups.

ing the study. There was a trend toward an increase in the hemoglobin and hematocrit in the CBC test after the TRT. No clinically significant changes were noted in the liver enzyme levels, coagulation parameters or results of the urine analysis during the treatment.

## Discussion

To our knowledge, this is the first dose-response analysis of TRT regarding the onset of the physical therapeutic effects. The general effects of TRT in female to male GID include; deepening of voice, increase in facial hair, cessation of menses, clitoral enlargement, vaginal atrophy, breast atrophy, muscular growth, acne and increased libido [3, 4]. Since the physical responses of deepening of voice, increase in facial hair and cessation of menses are the most commonly desired clinical effects of TRT, we focused on these physical changes. In this study, we analyzed three dose/frequencies of TRT and there were no statistically significant differences in the patient age and body weight between the three treatment groups. The results indicated that the onset of these physical changes was more prevalent in the group receiving the higher dose of testosterone after one month of TRT, and showed that there was a dose-dependent effect in the three treatment groups. On the other hand, after six months of TRT, most of the patients achieved the treatment responses, and there was no dose-dependency with regard to the cumulative percentage of patients with the therapeutic effects. It seems that the effect of TRT is dose-dependent at an early stage of TRT, but that eventually, various doses can achieve the desired effects after six months of TRT. Additionally, the design of this study was not randomized and the patients knew the dosage of TRT they received. In this point, there is a limitation of this study that the knowledge of self-dose may affect the assessment of the therapeutic effects and induce a bias potentially. A future randomized study including the quantitative evaluation of the therapeutic physical changes is planned to further elucidate the dose-dependency between the three dosing groups.

The average testosterone levels in the treatment groups reached around 700 ng/dL, which is comparable to the testosterone levels in normal males [9, 10]. Since these values indicate the average serum testosterone levels during TRT, it seems that the three doses of TRT all provide sufficient levels of serum testosterone for the virilization. Interestingly, there were no significant differences in the average levels of serum testosterone between the three groups. On the other hand, at one month after initiating TRT, the onset of the physical changes was more prevalent in the higher dosage group (250 mg every two weeks) in comparison to the lowest group (125 mg every two weeks). These results clearly indicate that a higher serum testosterone level is achieved by treatment with the higher dose of TRT. Based on these findings and a previous report [11], a diagram of the likely pharmacokinetics of intramuscular testosterone enanthate is shown in Fig. 6. As the average serum testosterone levels during TRT were similar in all three TRT treatment groups, it considered that the



Fig. 6 A diagram of the possible pharmacokinetics of intramuscular testosterone enanthate is shown for each dosage.

trough levels of serum testosterone were also similar in the three treatment groups. Furthermore the maximal level of testosterone should be different between the highest (250 mg every two weeks) and lowest (125 mg every two weeks) dosage groups. After the first month of TRT, this difference in testosterone pharmacokinetics between the three doses/intervals likely causes the dose-dependent therapeutic effects. Additionally, the average testosterone levels during TRT in all three groups were as high as the normal level in males, and this may explain why most of the patients had achieved the treatment responses, and why there was no dosedependent effect on the cumulative therapeutic outcomes at six months after starting TRT. With regard to the serum estradiol levels, we showed that TRT significantly decreased the levels in comparison to the pre-treatment levels. It is considered that testosterone stops the menses and directly suppresses the axis of female hormone signaling, including the serum estradiol. There were no significant differences in the average estradiol levels between the three treatment groups and this is consistent with the increase in the serum testosterone levels.

We demonstrated that the three doses/intervals of TRT evaluated in this study were safe and effective for the patients with female to male GID. We revealed that the early onset of the treatment effects of TRT is dosedependent, but that by six months after the initiation of TRT, there is no dose-dependency, and all three doses were effective. These results indicate that the higher dose of 250 mg every two weeks has an advantage for the patients who want to achieve a rapid therapeutic response, but that the lower dose of 125 mg every two weeks is also able to achieve the same results within six months of initiating treatment, and can reduce the costs of TRT. Although there were no significant side effects during the observation period of this study, it may be better to use the lower dose of 125 mg every two weeks to prevent the adverse effects in the patients receiving long term TRT. However, the dose of 250 mg every three weeks can be useful for the patients who prefer to visit the outpatient clinic at longer intervals. Thus, the information provided by this study is helpful for the patients and medical doctors to determine the optimal initial dose of TRT and to allow them to make changes in the continuous dosage of long term TRT.

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