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Testosterone replacement elevates the serum uric acid levels in patients with female to male gender identity disorder

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Abstract. Gender identity disorder (GID) results from a disagreement between a person's biological sex and the gender to which he or she identifies. With respect to the treatment of female to male GID, testosterone replacement therapy (TRT) is available. The uric acid (UA) level can be influenced by testosterone; however, the early effects and dose-dependency of TRT on the serum UA concentration have not been evaluated in this population. We herein conducted a dose-response analysis of TRT in 160 patients with female to male GID. The TRT consisted of three treatment groups who received intramuscular injections of testosterone enanthate: 125 mg every two weeks, 250 mg every three weeks and 250 mg every two weeks. Consequently, serum UA elevation was observed after three months of TRT and there was a tendency toward testosterone dose-dependency. The onset of hyperuricemia was more prevalent in the group who received the higher dose. We also demonstrated a positive correlation between increased levels of serum UA and serum creatinine. Since the level of serum creatinine represents an individual's muscle volume and the muscle is a major source of purine, which induces UA upregulation, the serum UA elevation observed during TRT is at least partially attributed to an increase in muscle mass. This is the first study showing an association between serum UA elevation and a TRT-induced increase in muscle mass. The current study provides important information regarding TRT for the follow-up and management of the serum UA levels in GID patients.

Key words: Gender identity disorder, Testosterone, Uric acid, Creatinine, Muscle

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GENDER IDENTITY DISORDER (GID) is a condition in which an individual suffers from a significant gender divergence between his or her actual biological sex and the gender to which he or she identifies. The concept of GID was introduced in the 20th century [1], and the number of patients diagnosed with this condition has been increasing in the past decade. Patients are treated with the cross-sex hormone in order to reduce the characteristics of their biological sex and develop the features of the opposite sex to which they feel they belong. Therefore, testosterone replacement therapy (TRT) is often administered in female to male

GID patients to promote male sex characteristics, such as a beard, body hair, increased muscle mass and a deeper voice [2-6]. However, testosterone administration also leads to side effects, including water and sodium retention, hypertension, increased erythropoiesis, an increased low-density lipoprotein (LDL) level, a decreased high-density lipoprotein (HDL) level, elevation of liver enzymes, induction of obesity and acne and emotional and psychiatric problems [2-6].

Uric acid (UA) is the end product of purine metabolism in humans. It is endogenously produced as a metabolite in the liver and primarily excreted by the kidneys and intestines [7]. Physiologically, the serum UA concentration increases with age and is higher in males than age-matched females [8]. The UA values are lower in females of childbearing age and, in postmenopausal females, increase to levels similar to those observed in males [7, 8]. On the other hand,

it is recommended to treat hyperuricemia (≥7.0 mg/dL) with dietary therapy and/or pharmacotherapy [9] because a higher level of serum UA is associated with an increased risk of disease, including gouty arthritis, cardiovascular disease, renal dysfunction, urinary tract stones, hypertension and diabetes mellitus [7, 8].

Recently, TRT has become more diverse, including a variety of formulations, doses and dose intervals. The most frequently used regimens include 100~250 mg of testosterone enanthate administered *via* intramuscular injection every two to three weeks [3, 4]. We previously reported the physical therapeutic effects of three TRT doses (125 mg every two weeks, 250 mg every three weeks and 250 mg every two weeks) [6]. We experienced that, when TRT is initiated, testosterone immediately affects the systemic metabolism in female to male GID patients. Although UA is a possible factor that can be influenced by testosterone administration [10-13], the early effects and dose-dependency of TRT on the serum UA concentration have not been previously determined. As for the effects of testosterone on the serum UA level, we herein report our experience using three TRT doses in the treatment of female to male GID.

Patients & Methods

GID clinic and patients

The GID clinic at Okayama University Hospital consists of four clinical departments: psychiatry, urology, gynecology and plastic and reconstructive surgery [14, 15]. Patients with female to male GID undergo a standard psychiatric evaluation to rule out major functional psychiatric disorders, such as schizophrenia, mood disorders and neurosis. The GID clinic provides genetic testing, diagnosis, counseling, hormonal therapy and plastic surgery, and all aspects of this study were approved by the Ethics Committee of Okayama University Hospital [6, 14, 15]. Between January 2002 and May 2011, a total of 160 Japanese patients were diagnosed with female to male GID and treated with testosterone replacement therapy (TRT) in the GID clinic. There were 107 patients in the low-dose group (125 mg every two weeks), 21 patients in the middle-dose group (250 mg every three weeks) and 32 patients in the high-dose group (250 mg every two weeks). The average patient ages in the three TRT dose groups were 24.9 ± 0.5, 26.1 ± 1.2 and 28.1 ± 1.0 years, respectively. The average body weight of all patients was 57.0 ± 1.0

Kg, and no significant differences were observed in body weight between the three TRT dosage groups.

Testosterone replacement therapy and follow-up

TRT was administered *via* regular intramuscular injections of testosterone enanthate in the outpatient clinic. The patients were divided into three groups based on the dose: 125 mg every two weeks, 250 mg every three weeks and 250 mg every two weeks. The patients selected their preferred dosage based on the visit interval and cost of treatment. The examination conducted at the initial visit included measurement of the serum UA concentration and serum creatinine concentration and hormonal tests of testosterone and estradiol. The remaining evaluations consisted of an interview, physical examination, chromosomal study, blood tests (CBC: complete blood cell count), biochemical tests (ALT: alanine aminotransferase, AST: aspartate aminotransferase), coagulation parameter measurements (PT: prothrombin time, aPTT: activated partial thromboplastin time), urinalysis and electrocardiogram. At three, six and 12 months after the initiation of TRT, all tests were repeated, except for the chromosomal study and electrocardiogram. Patients who discontinued the TRT, required changes in the dosage or received sex reassignment surgery during the follow-up period were excluded from this study. A retrospective study was carried out with respect to the testosterone dosage and serum UA concentrations.

Blood sample measurements

The serum samples were obtained in the afternoon at Okayama University Hospital and stored at −70°C until the analyses performed at the laboratory of SRL Inc. (Tokyo, Japan). The assays of serum testosterone and estradiol were performed using an electrochemiluminescence immunoassay (ECLIA), as previously described [6]. The serum testosterone level was measured as the total testosterone level; the standard value ranged from 131~871 ng/dL in males and 11~47 ng/dL in females. The standard value of the serum estradiol level ranged from 15~35 pg/mL in males and 20~550 pg/mL in nonpregnant females before menopause. Since many patients came to our clinic at their convenient timing of the blood sampling during TRT, we could not determine the exact timing of hormonal tests in relation to the latest testosterone injection. The serum UA level was measured using the enzymatic method, in which UA was oxidized by uricase.

Hyperuricemia was defined as a UA level of $\geq$7.0 mg/dL, which is commonly used to define a high serum UA concentration in the general population [9]. The level of serum creatinine was typically determined according to the enzymatic method.

Statistical analysis

The data are expressed as the mean ± standard error (S.E.), if not otherwise specified. Paired Student's t-test was performed for the statistical analysis between the pretreatment and posttreatment values. An analysis of variance (ANOVA) and Bonferroni/Dunn test was used for the statistical analysis between the dosage groups. We conducted a linear regression analysis to determine the correlation between the rate of serum creatinine increase and the rate of serum UA increase after three months of TRT. Differences were considered to be statistically significant at p <0.05.

Results

Changes in the serum UA levels after TRT

We first analyzed the serum uric acid (UA) concentrations of all patients before and during TRT. The levels were compared between the pretreatment values and the values obtained at three, six and 12 months after the initiation of TRT. Statistically significant elevation of the serum UA concentration was observed after three months of TRT, and this elevated level was maintained until at least one year of TRT (Fig. 1). We also conducted a dose-response analysis of TRT in terms of the effects on the serum uric acid concentration. We compared the three dosage groups (intramuscular injection of testosterone enanthate; 125 mg every two weeks, 250 mg every three weeks and 250 mg every two weeks). After three months of TRT, a significantly higher rate of serum UA increase was observed in the 250 mg every two weeks group in comparison to that observed in the other two groups (Fig. 2). We thus found that there was a tendency toward testosterone dose-dependency in serum UA elevation.

We next evaluated the onset of hyperuricemia induced by TRT, in which hyperuricemia was defined as a serum UA concentration of $\geq$7.0 mg/dL. No patients had hyperuricemia before TRT; however, patients with hyperuricemia were observed in all three dosage groups, even at three months after the initiation of TRT (Fig. 3). Patients with hyperuricemia were more prevalent in the high-dose group, thus indicating testosterone dose-dependency at the onset of hyperuricemia.
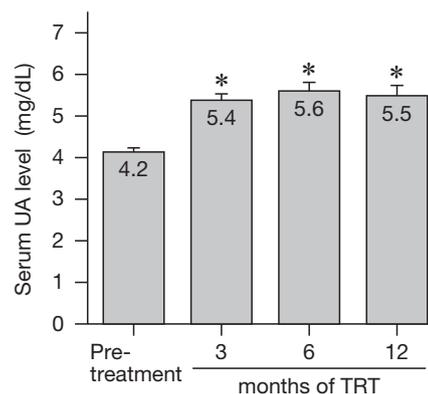

Fig. 1 The serum uric acid (UA) concentrations of all patients before and during TRT are shown. The levels are compared between the pretreatment values and the values obtained at three, six and 12 months after the initiation of TRT. A significant difference (*p<0.05) was observed between the pretreatment levels and the levels at three, six and 12 months after TRT initiation.
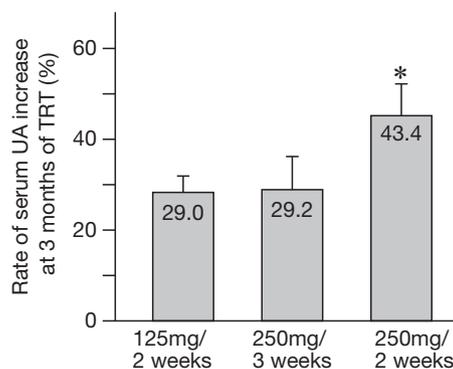

Fig. 2 The rates of serum UA increase after three months of TRT in the three dosage groups are shown. A significant difference (*p<0.05) was observed between the group treated with 250 mg every two weeks and the other dosage groups.
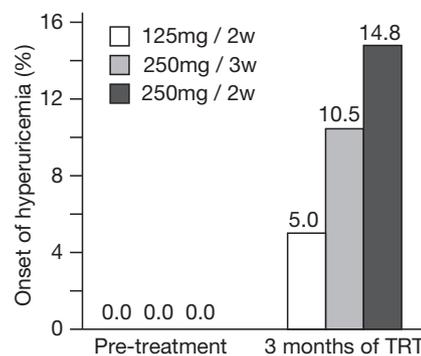

Fig. 3 The percentages of patients with onset of hyperuricemia at three months after the initiation of TRT in the three dosage groups are shown. Hyperuricemia was defined as a serum UA concentration of $\geq$7.0 mg/dL.

Hormonal parameters

The serum testosterone level at three, six and 12 months after the initiation of TRT was averaged and used in each patient. Since the hormonal examinations of each patient were conducted on different days after the latest testosterone injection, the serum testosterone values represent the average level of testosterone observed during TRT. The average testosterone level significantly increased compared to the pretreatment level following TRT in all three dosage groups (Fig. 4A). The testosterone level reached 700~800 ng/dL in each treatment group, and there were no significant differences in the testosterone levels between the groups. On the other hand, the serum estradiol levels significantly decreased during TRT in comparison to the pretreatment levels. Similar to the levels of testosterone, the values of estradiol indicated the average serum estradiol level during TRT in each dosage group. There were also no significant differences between the three dosage groups in terms of the average estradiol level (Fig. 4B).

Correlation between increases in serum creatinine and serum UA

In order to analyze the correlation between the increase in serum creatinine and the increase in serum UA, we conducted a linear regression analysis among the entire population of patients. We evaluated the rate of serum creatinine increase (%) and the rate of serum UA increase (%) after three months of TRT. A scattergram and linear regression analysis showed a statistically significant positive correlation between the increase in serum creatinine and the increase in UA (regression-coefficient, 0.59; 95% CI, 0.296-0.883; p = 0.0001) (Fig. 5). Since the level of serum creatinine represents individual muscle volume and has been demonstrated to be a reliable biomarker of skeletal muscle mass [16], the results indicate a correlation between an increase in muscle mass and serum UA elevation at the examined time points of TRT.

Side effects of TRT

No significant adverse events were observed in any of the dosage groups during this study. All patients were satisfied with the therapeutic effects of TRT, and no discontent was observed during treatment. TRT did not induce adverse symptoms or diseases due to hyperuricemia in any of the dosage groups. There was a tendency toward increased values of systolic and diastolic
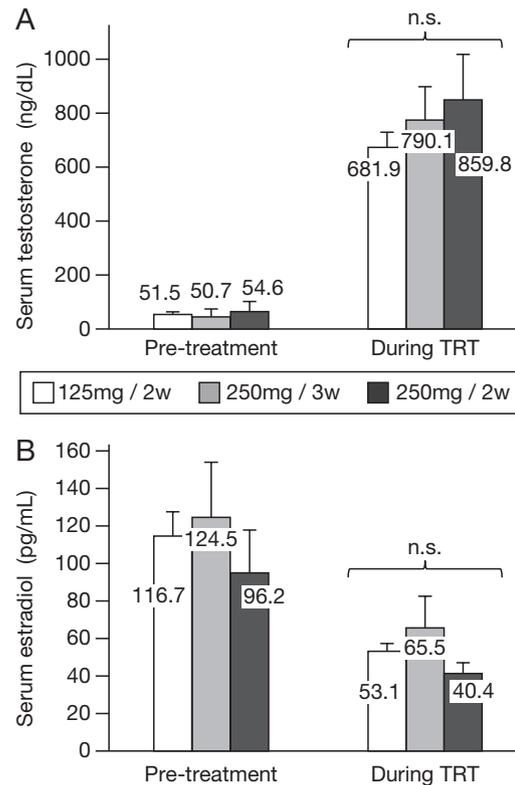

Fig. 4 The serum concentrations of testosterone (A) and estradiol (B) before and during TRT are shown. The average levels of testosterone and estradiol during TRT were calculated in each patient. The bar graph indicates the average concentrations of testosterone and estradiol during TRT in each dosage group. [n.s.] indicates no significant differences between the three dosage groups.
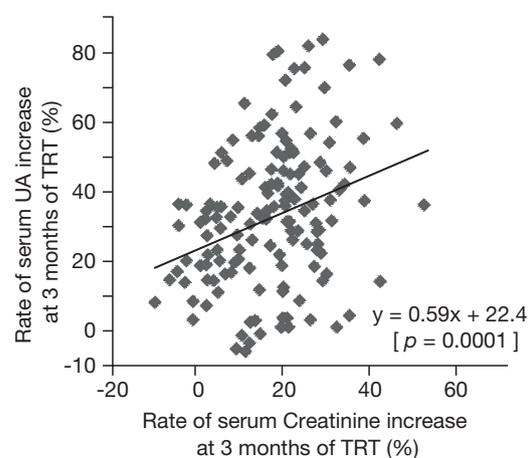

Fig. 5 The correlation between the increase in serum creatinine and the increase in serum UA was analyzed in all patients. The rate of serum creatinine increase (%) and the rate of serum UA increase (%) after three months of TRT were plotted in a scattergram. A significant positive correlation was observed between these two parameters in a linear regression analysis.

blood pressure during the observation period; however, no definitive cardiovascular diseases were clinically relevant. There was a trend toward increased levels of red blood cell (RBC), hemoglobin and hematocrit on the CBC examinations conducted during TRT. No clinically significant changes were observed in the levels of liver enzymes or coagulation parameters during the treatment period (Table 1).

Discussion

Regarding the serum UA concentration after TRT, to our knowledge, this is the first study to conduct an early phase and dose-dependent analysis of female to male GID patients. The serum UA levels were found to be upregulated after three months of TRT, and the elevated levels were maintained until at least one year of TRT. We showed that there was a tendency toward testosterone dose-dependency in serum UA elevation. We also demonstrated that the onset of hyperuricemia was more prevalent in the high-dose group. As shown in previous studies, maximal serum testosterone levels are achieved immediately after testosterone injection, and early testosterone levels are TRT dose-dependent [6, 17]. Therefore, the higher serum testosterone concentration induced by a higher dose of TRT causes dose-dependent serum UA upregulation. On the other hand, as for the average values of serum testosterone and estradiol in the three TRT dosage groups, there was no dose-dependent elevation or suppression in any group. The absence of dose-dependency in these hormone levels is consistent with the findings of a previous study, and the serum hormone values measured at variable times of sampling in relation to the last testosterone injection did not reflect the TRT dose [6].

Several authors have previously examined the influence of testosterone and estradiol on the serum UA level [10-13]. A study of patients undergoing male to female gender reassignment reported that estrogen replacement therapy reduces the serum UA concentration and increases urinary UA excretion [10]. Estrogen replacement therapy also reduces the serum UA concentrations in hyperuricemic postmenopausal females [11]. With respect to TRT, only two reports have shown that testosterone treatment for female to male gender reassignment leads to increased serum UA concentrations and reduced renal excretion of UA [12, 13]. Our current study also demonstrated serum UA upregulation induced by testosterone administration. This is the first study to show TRT dose-dependent effects in this population. These results suggest that an increased serum testosterone level and/or a decreased serum estradiol level induced by TRT results in serum UA elevation. As for the mechanisms underlying the TRT-induced increases in the serum UA concentration, it seems likely that sex hormones primarily influence the serum UA concentration *via* changes in renal UA excretion [10-13]. Testosterone and estradiol exhibit suppressing and enhancing effects, respectively, on renal UA excretion, and this theory also explains age and sex differences in the serum UA levels.

To further clarify the mechanisms by which TRT elevates the serum UA concentrations in female to male GID patients, we examined the association between increases in serum UA and other blood or physical examination parameters. As for the changes of serum testosterone level and body mass index, there was no significant association with the increases in serum UA level (data not shown). Notably, we demonstrated a positive correlation between increased levels of serum UA and serum creatinine after three months of TRT, indicating that the serum UA elevation observed in the patients was at least partially attributed to an increase in muscle mass during the early phase of TRT. We thus found a novel association between serum UA elevation and an increase in muscle mass in patients with TRT. As for the manner in which an increase in muscle mass upregulates the serum UA concentration, various the-

Table 1 The laboratory data of pretreatment and after three months of TRT.

Parameter	Pre-treatment	After 3 months of TRT	*p* value
RBC ($\times 10^6/mm^3$)	4.36 ± 0.03	4.89 ± 0.03	< 0.0001
Hemoglobin (g/dL)	13.3 ± 0.08	14.2 ± 0.12	< 0.0001
Hematocrit (%)	39.5 ± 0.23	42.8 ± 0.33	< 0.0001
ALT (IU/L)	16.6 ± 1.33	16.6 ± 0.72	0.93
AST (IU/L)	18.6 ± 0.85	20.5 ± 0.81	0.11
PT (seconds)	12.5 ± 0.68	11.6 ± 0.11	0.13
aPTT (seconds)	33.2 ± 0.32	32.8 ± 0.49	0.16

ories are conceivable in terms of muscle metabolism. The anabolic effects of testosterone administration on muscle mass have been clearly demonstrated in female to male GID patients as well as the general male population [2-5]. In addition, muscle mass is considered to be a major source of purine, which induces serum UA upregulation [18-20]. During rapid increases in muscle mass, TRT induces the active replacement of old muscle with newly regenerated muscle. This process is likely to require compensatory muscle cell depletion and can increase the provision of nucleic acids and purine to the liver, resulting in increased production of UA. Hence, the rapid increase in muscle mass is accompanied by serum UA upregulation due to activated purine metabolism in these patients. In addition, it is possible that increased muscle mass leads to greater adenosine triphosphate (ATP) consumption in order to obtain more energy. Increased ATP metabolism causes the release of purine intermediates in the muscle and induces serum UA elevation. Based on our experience, once TRT is initiated, some patients with female to male GID dramatically achieve a muscular body through heavy exercise and muscular labor. Therefore, we cannot rule out the possibility that heavy exercise results in the accumulation of injured muscle cells, increasing the metabolism of nucleic acids and purine, which then elevates the serum UA concentration [21, 22].

The onset of gout induced by TRT is rare; however, a case report of the development of gout following therapy for female to male gender reassignment has previously been reported [13]. Previous research also indicates an association between the UA level and cardiovascular morbidity and suggests that mortality is greater in females than in males in middle life [23]. Although no adverse symptoms or diseases due to hyperuricemia were observed in any of the dosage groups during this study, TRT substantially elevates the serum UA levels in female to male GID patients. We demonstrated that the early onset of hyperuricemia is induced by TRT, even though the treatment period was short at three months and the patients were young and intrinsically female. If older patients are treated with long-term TRT, the progression of serum UA elevation and other metabolic effects may increase the risk of diseases, such as gout and cardiovascular disease [7-9, 23]. Moreover, it is possible that TRT accelerates the onset of hyperuricemia and gout in combination with underlying genetic risks of these conditions. Therefore, serum UA elevation is an important side effect to be addressed, and the medical team should be alert to the possibility of excess hyperuricemia from the early phase of TRT. The information provided here is available for both patients and medical doctors to determine the optimal initial dose of TRT and to follow-up and manage the serum UA level.

Acknowledgements

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