Gradually increasing ethinyl estradiol for Turner syndrome may produce good final height but not ideal BMD

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Abstract. Estrogen replacement therapy in Turner syndrome should theoretically mimic the physiology of healthy girls. The objective of this study was to describe final height and bone mineral density (BMD) in a group of 17 Turner syndrome patients (group E) who started their ethinyl estradiol therapy with an ultra-low dosage (1-5 ng/kg/day) from 9.8-13.7 years. The subjects in group E had been treated with GH 0.35 mg/kg/week since the average age of 7.4 years. The 30 subjects in group L, one of the historical groups, were given comparable doses of GH, and conjugated estrogen 0.3125 mg/kg/week −0.3125 mg/day was initiated at 12.2-18.7 years. The subjects in group S, the other historical group, were 21 patients who experienced breast development and menarche spontaneously. Final height (height gain < 2 cm/year) in group E was 152.4 ± 3.4 cm and the standard deviation (SD) was 2.02 ± 0.62 for Turner syndrome. The final height in group L was 148.5 ± 3.0 cm with a SD of 1.30 ± 0.55, which was significantly different from the values for group E. The volumetric BMD of group S (0.290 ± 0.026 g/cm$^3$) was significantly different from that of group L or E (0.262 or 0.262 g/cm$^3$ as a mean, respectively). This is the first study of patients with Turner syndrome where estrogen was administered initially in an ultra-low dose and then increased gradually. Our estrogen therapy in group E produced good final height but not ideal BMD.

Key words: Ethinyl estradiol, Turner syndrome, Final height, BMD (bone mineral density)

TURNER SYNDROME (TS), originally described by Turner et al. [1], is one of the most common sex chromosome aneuploidies, with an incidence of 1 case per 2,000-2,500 females [2]. The two main treatable symptoms are short stature and delayed puberty. Short stature, observed in more than 95% of pediatric TS outpatients [3, 4], is a very common feature of this condition and affects the patients’ QOL. The efficacy and safety of GH treatment for this symptom is well established [5-7] and early initiation of GH treatment may be beneficial [8, 9].

Although there is no doubt regarding the efficacy and physiological significance of estrogen replacement therapy for delayed puberty, when the therapy should be started, and in what increments it should be administered, are unclear. Estrogen replacement for TS should ideally mimic a physiology of healthy girls; hence treatment should begin with an ultra-low dosage and gradually increase. Nonetheless, no studies to date have examined the merits of this kind of therapy. Indeed studies on estrogen replacement therapy for pediatric cases of TS are rare.

Recently, Quigley et al. reported in a randomized-control study that low dose ethinyl estradiol administration (EE$_2$; 25 ng/kg/day) starting at the age of 5-8 years improved final height [10, 11]. They focused on estrogen replacement therapy in TS, and analyzed only height data without addressing bone mineral density (BMD), which is also estrogen-dependent. Furthermore, the starting dose of 25 ng/kg/day was still a pharmacological dosage and the age of the initiation of EE$_2$, from 5-8 years, was relatively young in comparison with the norm for the Caucasian population. The standard adult EE$_2$ dose is 400 ng/kg (roughly 20 mg/day), and average breast development starts around the age of 10-11 years among the Caucasian population.
We administered EE\textsubscript{2} to 17 patients with TS (Early-group; group E) to imitate physiological pubertal development. The dosage and age at the initiation were mostly 1–2 ng/kg/day and 11.6 years on average, respectively. The dosage was increased gradually then followed by the administration of classical conjugated estrogen (Premarin\textsuperscript{R}). Progesterone was added to induce menarche. The method used for group E was designed to mimic the normal physiological process in terms of the low initial EE\textsubscript{2} dose and subsequent gradual increase.

Here we show the results of the analysis of final height and volumetric BMD (vBMD) in this group. The comparison to group E was made by using the two historical groups, namely: group L, in which estrogen was started late with a larger dosage and group S, in which puberty occurred spontaneously. The final height of group E was significantly greater than that of groups L and S, whereas the vBMD of group E was significantly lower than that of group S.

**Subjects and Methods**

This study was a cohort study conducted at a tertiary hospital in Tokyo, Japan. The diagnosis of TS was made by G-band analysis of the peripheral lymphocytes. The minimal lymphocyte cell number for the diagnosis was 30.

**Subjects**

The subjects were 78 patients with TS who are currently being followed or were followed at our institution without interruption until final height was achieved (as defined by height velocity of less than 2 cm per year). Ten of these patients were excluded due to poor compliance such as a frequency of GH injection of less than 50% (N=3), absence of adequate clinical information such as the method of estrogen increase (N=3), or complications potentially affecting height and BMD such as coarctation of the aorta, anorexia nervosa, and scoliosis (N=4). The remaining 68 patients comprised the final subject pool, which included group E, within-subjects design group (see below).

The subjects other than those in group E was subdivided into groups L and S, the two historical groups, as described above. The definitions of these three groups are given below.

**Group E (Designed group treated with ultra-low dose EE\textsubscript{2})**

The subjects in this group were 17 females (see in Table 1), who had been treated with GH 0.35 mg/kg/week (6 or 7 days/week) since the average age of 7.4 years with a range of 1.5-13.7 years. EE\textsubscript{2} was stared at 1-5 ng/kg from the age of 9.8-13.7 years; 15 and 2 patients in group EE\textsubscript{2} started in the dose of 1–2 and 5 ng/kg, respectively. FSH concentration was ascertained to be greater than 10 mIU/mL before or at the initiation of EE\textsubscript{2}. FSH 10 mIU/mL or higher is a hallmark of the future absence of a spontaneous and regular menstrual cycle in TS as we have already reported [12].

The initial regimen of EE\textsubscript{2} administration (1, 2, 4-5 ng/kg/day) was planned with a gradual increase every 6-8 months except in the 3 cases mentioned below.

<table>
<thead>
<tr>
<th>Table 1 Demographic features of the three groups</th>
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<tr>
<td>Groups (N)</td>
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<td>E (17)</td>
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<td>N of 45,X $^a$</td>
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<td>N of 45,X/46,XX and 45,X/47,XXX $^a$</td>
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<td>Age of GH Tx (years) $^b$</td>
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<td>Ht of GH Tx (T-SD $^{***}$ for ref. 13)</td>
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<td>Age of estrogen Tx (years)</td>
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Number and mean are shown. $^a$ Other types of the karyotype than 45,X, 45.X/46,XX, and 45.X/47,XXX were consistent with the diagnosis of Turner syndrome, such as 45.X/46,Xidic(X) and 46,Xidic(X). $^b$ GH treatment was administered in 14 out of 21 patients in this group. Estrogen replacement therapy was started at the age of 15-17 years in 7 out of 21 patients in this group. $^{***}$ There was a significant difference in age at the initiation of GH treatment between groups E and L (see the main text). $^{****}$ T-SD indicates Turner syndrome specific SD.
of this period was 1.66 ± 0.20 years (range 1.21~1.89; N=14), respectively, after excluding the 3 cases in which the duration of this period was just 0.50-0.60 years. The duration of treatment in these 3 subjects was short, since the initial dose was 5 ng/kg in 2 of the 3 cases while the EE2 dosage was increased more rapidly in 1 case in compliance with the patient’s wishes. After this initial period of EE2 administration, we further increased the dosage like 8-10, 20-25, 50, 100 ng/kg/day each 6 ± 2 months.

Finally the estrogen was switched from EE2 to conjugated estrogen (CE) as 0.3125 and 0.625 mg/day, each for 3-12 months. Thereafter, menarche was induced at the age of 13.8-16.8 years (N=17, mean and SD: 15.7 and 1.0 years) by using CE 0.625-1.25 mg/day and medroxyprogesterone acetate 5-10 mg/day.

The mean and SD in length from the initiation of EE2 to the onset of menarche was 4.2 ± 0.8 years (range 2.8~5.4; N=17). This method of estrogen therapy in group E was started at our hospital after 2000. The current mean age at onset of menarche among Japanese girls is around 12-13 years.

Group L (One of the two historical groups)

The subjects in this historical group were 30 females (see Table 1) who were treated with GH like those in group E, except that the treatment was started later at the age of 10.5 ± 3.1 years (range; 3.7~13.8). CE was started at the age of 12.2-18.7 years. The minimal initial dose was 0.3125 mg/day, once a week. All FSH levels were higher than 10 mIU/mL in this group as well, before or at the initiation of estrogen treatment. When the age at the initiation of CE was relatively early, the CE dose was increased gradually. For example, 0.3125 mg was initially administered once a week, then the same dose was administered twice a week, 3 times a week, every day, and so on; then the dosage was increased to 0.625 mg daily. When the age of the initiation was relatively late, the initial dose was set at 0.3125-0.625 mg/day. Menarche in group L was induced relatively late by present standards at the age of 14-19 (mean; 17.7) years after maintaining a dosage of 0.625 mg of CE at least for half a year. The estrogen therapy in this group was initiated at our institution before 2000.

Group S (The other of the two historical groups)

The subjects in this group were 21 females who experienced breast development and menarche spontaneously without estrogen replacement. Menstruation continued for at least 1 year after onset of menarche in this group. GH was administered to 14 out of the 21 subjects in this group. Estrogen replacement therapy was started at the age of 15-17 years in 7 out of 21 patients in this group.

Methods

Height

Final height, as defined above (height velocity < 2 cm/year), was calculated in centimeters with the SD for both healthy Japanese females and TS patients [13].

Bone Mineral Density (BMD)

First, the BMD was assessed in units of g/cm² using a standard dual-energy X-ray absorptiometry machine (DXA; Hologic, Bedford, MA, USA) at the lumbar vertebrae (L2-4). Second, the results were transformed into volumetric BMD [14]. These vBMD data were not obtained from 4 subjects, two in group L and one in each of groups S and E, since their original BMD data had not been kept in our institution. If the measurement was repeated more than twice, the first value obtained after achievement of final height was used. vBMD data were taken after 18 years old except in 6 patients in group E.

Statistics

Graphpad Prism version 6 (GraphPad Software, Inc.) was used for statistical analysis. Mann Whitney test was used to compare final height data (cm), Turner specific height SD at the age of GH initiation, and age of the initiation of GH treatment between groups E and L. This test was also performed to compare the vBMD data within group E, L, and S. 1-way ANOVA was used to compare the mean BMI for each of the groups E, L, and S. A 2-tailed p value of less than < 0.05 was considered significant.

Ethics

This study was approved by the ethical committee in our institution (H27b-135). Informed consent was obtained from all the guardians or subjects in this study.

Results

The demographic features of the patients in the three groups are shown in Table 1. A significant difference was observed in age at the start of GH treatment between groups E and L (E < L; p=0.002). However, SD of height for TS at the initiation of GH treatment was not significantly different between groups E and L (p=0.55).
The final height of group E (N=17) was 152.4 ± 3.4 cm, which was +2.02 ± 0.62 SD for TS (Table 2 and Fig. 1). The most optimal final height in group E was 159.2 cm in 1 patient with the target height of 157 cm. This final height corresponds to +3.3 SD and 0.2 SD for TS and healthy Japanese females, respectively. In this particular patient, BMD at the final height was -0.1 SD, which roughly matched 0.2 SD, the final height SD for healthy Japanese females. Her GH, estrogen, and medroxyprogesterone acetate were started at the ideal timing of age 2.2 years, 9.8 years, and 13.8 years, respectively.

The final height in group L was 148.5 ± 3.0 cm with a SD of +1.30 ± 0.55 for TS (Table 2 and Fig. 1). There was a significant difference in height (cm) between groups E and L (E > L; p=0.0002).

Considering the significant difference in age at the commencement of GH treatment between groups E and L as explained above, we compared the final height data of these two groups after excluding 15 subjects from group L, who began GH treatment at the age of 11 years or more. In the remaining 15 subjects in group L, the age at commencement of GH treatment (8.0 years as mean) and Turner-specific height SD scores at commencement (0.17 SD as mean) were not significantly different from the corresponding values in group E (7.4 years and 0.30 SD as mean). The mean final height of these 15 subjects in group L (group L-15) was 149.2 cm, and their height data were still significantly different from those of group E (E>L, p=0.0052). Even if 10 subjects who began GH treatment later than the others were excluded from group L, a similar significant difference would be obtained (data not shown).

Table 3 shows the final height data from well-established research, both from Japan [15-17] and other countries [5-7, 11], including two randomized-control trials [7, 11]. The data obtained with the same or similar GH doses are shown for comparison. The difference between the final height in our group E and the historical mean for untreated Japanese TS patients [13] was 11.1 cm. The differences in the final height compared to that of the placebo group in the two randomized-control trials [7, 11] were 4.7 and 6.5 cm. Those compared to the mean historical height without GH [13, 18] were 3.5, 7.1, 8.0, 8.3 and 10.7 cm [5, 6, 15-17].

vBMD results for groups E, L, and S are shown in Table 4 and Fig. 2. There was no significant difference in vBMD between groups E and L, whereas the vBMD data in group S were significantly different from those in either group E or L (p=0.0056 or p=0.0019, respectively). A comparison of BMD Z-scores obtained from the raw data (g/cm²) and height SD scores showed no significant difference in either group E or L (data not shown), suggesting that the BMD in either group E or L was height-matched. On the other hand, a significant difference (p<0.0001) was observed in group S between the two SD scores (N=21, BMD Z-score -0.8 ± 0.7, height SD score -2.74 ± 1.03).

**Table 2** Final height and body mass index (BMI) for groups E, L, and S

<table>
<thead>
<tr>
<th>Groups (N)</th>
<th>E (17)</th>
<th>L (30)</th>
<th>S (21)</th>
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<tbody>
<tr>
<td>Final height (T-SD)</td>
<td>2.02 ± 0.62</td>
<td>1.30 ± 0.55</td>
<td>0.42 ± 0.99</td>
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<tr>
<td>Final height (cm)</td>
<td>152.4 ± 3.4</td>
<td>148.5 ± 3.0</td>
<td>143.6 ± 5.5</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 4.5</td>
<td>22.4 ± 2.9</td>
<td>23.4 ± 4.2</td>
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The mean and SD are shown. T-SD indicates Turner syndrome specific SD. A significant difference as described in the text. BMI values were not significantly different among the three groups.

**Fig. 1** Final height (FH) in the 3 groups (E, L, and S groups) The mean and SD are shown as bars.

**Discussion**

This is the first study of patients with TS where estrogen was administered initially in an ultra-low dose and then increased gradually. The initial dose of EE2, 1-5 ng/kg in group E, was extremely low even in comparison with the dosage of 25 ng/kg reported in a previous study [10, 11]. Additionally, this is the first study...
of TS, where BMD was also taken into consideration in terms of the timing and method of exogenous estrogen administration.

GH and estrogen treatments administered to group E were beneficial in terms of height prognosis than comparative treatments given to group L, as seen in the significant difference in final height between these two groups. Although the subjects’ age at the start of GH treatment was significantly lower in group E than in group L, the SD scores for height were not significantly different between these two groups at the start of treatment, indicating no difference in projected height before GH treatment between these two groups. Indeed, adult height data calculated after the exclusion of the 15 older patients in group L were still significantly different from those of group E as described. As far as we know, the final height data in E group were one of the best among the data published so far in Japan and elsewhere for comparable doses of GH (Table 3).

Our EE2 therapy did not have any adverse effect on final height. Any kind of early initiation of estrogen in TS may enhance bone maturation, theoretically deteriorating the prognosis for final height. It is well known that not only BMD acquisition but also bone maturation is estrogen-dependent [19]. In a recently published report by Quigualy et al. [10], where the initial dose of EE2 was 25 ng/kg, breast development was observed in some of the patients. This pubertal

<table>
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<th>Table 3: Final Height in Turner syndrome patients after estrogen replacement and GH treatment at similar doses (without anabolic steroid)</th>
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# These two studies were performed as randomized clinical trials. 
*** Not described. 
##### Based on reference source 13 or 18.

| Table 4: Volumetric Bone mineral density (vBMD as g/cm$^3$) at final height |
|-----------------|-----------------|-----------------|-----------------|
| Groups (N)      | E (16)          | L (28)          | S (20)          |
| Mean            | 0.262 $^\#$    | 0.262 $^\#$    | 0.290           |
| SD              | 0.027           | 0.033           | 0.026           |

The mean and SD are shown. $^\#$ A significant difference against group S as described in the text.

Fig. 2: Volumetric BMD (vBMD) in the 3 groups (E, L, and S). The mean and SD are shown as bars.
development might theoretically have accelerated bone aging, resulting in a decrease in final height, as indeed it did in group S, which experienced early estrogen-exposure presumably resulting in early bone maturation and a lower final height. Unfortunately, bone age assessments were not performed regularly in group S.

The higher vBMD values contrasting with the lower final height in group S, compared with the corresponding values for group E, should be noted. Endogenous estrogen production in most girls presumably begins well before the initial signs of pubertal development, i.e., breast development, become evident [20]. Therefore, the vBMD findings observed in group S, which were significantly greater than those in group E, may indicate that estrogen administration in group E should have been started earlier than done in this study in order to achieve adequate BMD in patients with TS who are not expected to experience regular menstruation. One of our previous studies reported a decrease in actual BMD as measured by quantitative computed tomography in TS patients not only during, but also before puberty [21], as well as in other groups [22, 23].

Other potential estrogen derivatives that could be administered in increasing amounts are estradiol (either orally or transdermally) and CE. CE is the standard treatment at our institution, as seen with group L. An ultra-low dose at initiation followed by a gradual increase, by using either estradiol or CE, might have similar results to those seen in group E. It is still unknown whether estradiol, in particular via transdermal administration, should be used as the first-line treatment to induce puberty in TS patients. In adults, estradiol via transdermal administration was reported to be more beneficial for preventing thrombus; this type of administration, by bypassing the liver (avoiding the first-pass effect) and decreased clotting factors, minimizes the risk of thromboembolic events [24, 25]. EE2 was used for the estrogen treatment of group E in the present study in order to enable comparison with other historical studies where EE2 was used [10, 11, 26].

This study has a number of limitations. First, it was conducted only at one institution. Second, groups L and S in this study were not designed at all and the analysis in these two groups was totally retrospective. The design of group E was not very strict; for example, the initial dose of EE2 ranged from 1 to 5 ng/kg. However, our manuscript is the first report of estrogen therapy for Turner syndrome starting with an ultra-low dose, and is therefore of clinical relevance. Thirdly, the mean age (11.6 years) of initiation of EE2 in group E was still high, considering the fact that the mean age at the start of breast development is roughly 10 years among healthy Japanese females. Given all these limitations, a further study designed prospectively for multiple centers, is needed.

In conclusion, our titration method of estrogen therapy, starting around or below 1% of the adult dose, and then gradually increasing it over the ensuing years, achieved favorable final height but not ideal BMD in the patients with TS. These findings may have an impact on not only patients with TS but also those with other types of childhood hypogonadism. Further studies are required to better understand the effect of the early initiation of estrogen, in particular for the improved acquisition of BMD.

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Disclosure Statement
Nothing to declare.

Author Contributions
DA, MI and JI equally contributed to patient care and MF, MH and HY similarly contributed to data management. MG reviewed the manuscript. YH designed this study and wrote the manuscript.

References


