ORIGINAL

Efficacy of estrogen replacement therapy (ERT) on uterine growth and acquisition of bone mass in patients with Turner syndrome

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Abstract. Estrogen replacement therapy (ERT) is necessary for uterine development and bone mass acquisition in women with Turner syndrome (TS) suffering from ovarian insufficiency. However, adequate ERT regimens have not yet been established. The aim of this study was to evaluate the efficacy of ERT for both uterine development and bone mass acquisition. One hundred TS patients from Yokohama City University Hospital (88 with primary amenorrhea (PA) and 12 patients with spontaneous menstrual cycles (MC)) were enrolled after obtaining consent. Clinical profiles, uterine length (UL) measured by ultrasonic examination, and bone mineral density (BMD) of the lumbar vertebrae (L2–4) assessed by DEXA were evaluated. At the time of the first visit, the ULs of patients in the PA group were significantly shorter than those in the MC group. After receiving ERT, there were no significant differences in UL between patients with PA and MC. Forty-seven patients for whom the ERT initiation age was known were investigated to clarify the influence on BMD. The results showed that the BMD in the late initiation (18 years or older) group at the latest visit ($0.770 \pm 0.107 \text{ g/cm}^2$; $n = 16$) was significantly lower than that in the early initiation (under 18 years) group ($0.858 \pm 0.119 \text{ g/cm}^2$; $n = 21$) or the MC group ($0.941 \pm 0.118 \text{ g/cm}^2$; $n = 10$). No significant differences were seen between the early initiation and MC group. ERT was effective in increasing UL and BMD. However, early initiation of ERT is necessary to increase BMD.

Key words: Turner syndrome, ERT, Uterine growth, Bone mineral density, Puberty

TURNER SYNDROME (TS) occurs in 0.04% of female infants. This condition is due to loss or structural abnormality of an X chromosome. There are various complications associated with this condition; such as short stature, ovarian dysfunction, thyroid dysfunction, and glucose intolerance [1, 2]. Since primary amenorrhea due to ovarian dysfunction is a common complication in women with TS, ERT is necessary for induction of puberty and acquisition of bone mass [3].

Although there are some guidelines [2] or reports demonstrating the efficiency of ERT [4-6] in inducing puberty or increasing bone mass individually, little is known about the effects of ERT on both attributes in a single study, in adolescents with TS [7, 8].

To evaluate the efficacy of ERT both in inducing puberty and increasing bone mass in TS patients, a retrospective analysis in TS patients in our department was conducted.

Materials and Methods

The subjects comprised 100 patients with TS followed up in our department up to September 2013. There were 88 patients in the primary amenorrhea (PA) group and 12 in the spontaneous menstrual cycle (MC) group. The clinical profiles of the patients were examined with their written informed consent.

Medical history and clinical profiles of the patients were extracted from their medical records.

BMD of the lumbar vertebrae (L2–4) was measured by dual-energy X-ray absorptiometry (DEXA). UL was measured by ultrasonic examination.

ERT was initiated or continued in the PA group. In cases where it was introduced during adolescence, con-

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jugated estrogen (CEE) was started at one-eighth of the adult dose at 12–15 years of age and gradually increased double dose every six months according to the guidelines. When the adult dose had been administered for six months or breakthrough bleeding was recognized, the therapy was changed to cyclic estrogen and progesterone therapy. With regard to the adult dose, a monthly dose of CEE (0.625 mg/day for 21 days) together with medroxyprogesterone acetate (MPA: 5 mg/day for 10 days) was used in almost all cases.

The Kruskal-Wallis test and the Mann-Whitney U test were used for statistical analysis. A *P*-value of less than 0.05 was considered statistically significant.

Results

Patient characteristics are shown in Table 1. There were no significant differences in age, age at first visit, number of past GH therapy, or BMI among the groups. In the MC group, no patients with monosomy were observed and mean height was significantly shorter than that of the PA group. The mean age at initiation of ERT in the PA group was approximately 21 years on average and the mean age of menarche in the MC group was approximately 13 years. Thus there was an approximately eight-year difference between the two groups.

At the time of the first visit, the UL of patients who had already received ERT (47.6 ± 14.3 [mean $\pm$ SD] mm; $n = 72$) was longer than that of patients who had not received ERT (38.0 ± 10.2 mm; $n = 16$) in the PA

group. But, there was no significant difference. The UL of both groups (aforementioned) was significantly lower (previous ERT (-): $P = 0.001$, previous ERT (+): $P = 0.005$) than that of patients with MC (62.0 ± 11.3 mm; $n = 12$) (Fig. 1A).

After receiving ERT, the ULs of patients with and without previous ERT significantly increased to 59.7 ± 12.4 mm and 60.7 ± 6.3 mm, respectively (Fig. 1B). As a result, there were no significant differences in UL between patients with or without previous ERT and patients with MC (Fig. 1B).

The BMD of patients with previous ERT at first visit (0.809 ± 0.120 g/cm²; $n = 72$) was significantly higher than that of patients without previous ERT (0.743 ± 0.126 g/cm²; $n = 16$). However the BMD of both groups was significantly lower than that of patients with MC (0.970 ± 0.129 g/cm²; $n = 12$) (Fig. 2A). After receiving ERT, the BMD of patients with and without ERT increased to 0.795 ± 0.107 g/cm² and 0.832 ± 0.119 g/cm², respectively. On the other hand, there were no significant changes in the BMD of patients with MC (0.953 ± 0.118 g/cm²). However, the BMD of the PA groups remained significantly lower than that of patients with MC (Fig. 2B).

To clarify how the age of initiating ERT influenced bone mass acquisition in Turner syndrome, 47 patients referred from Kanagawa Children's Medical Center (KCMC) were investigated because the regimen of ERT used at that institution had been standardized.

The patient characteristics are shown in Table 2.

Table 1 Characteristics of the patients and comparison of various values between PA group (with and without previous ERT) and MC group

	Primary amenorrhea ($n = 88$)		Menstrual cycle ($n = 12$)	<i>P</i>
	Previous ERT (-) ($n = 16$)	Previous ERT (+) ($n = 72$)		
Age (years)	31.8 ± 9.1 (17–58)	32.0 ± 7.4 (18–49)	28.9 ± 6.6 (18–40)	NS
Age at initial evaluation	23.6 ± 8.9 (15–50)	26.1 ± 6.6 (14–41)	23.2 ± 5.0 (18–30)	NS
Height (cm)	147.2 ± 7.9 (130.0–163.0)	145.6 ± 5.1 (132.0–158.0)	141.1 ± 6.0 (130.0–149.5)	0.047
BMI (kg/m ²)	22.2 ± 4.8 (15.8–36.0)	21.9 ± 3.6 (15.0–33.6)	23.0 ± 3.2 (19.0–29.0)	NS
Karyotype	Monosomy 6 Structural abnormality 5 Mosaic 4 Unknown 1	Monosomy 11 Structural abnormality 19 Mosaic 30 Unknown 9	Structural abnormality 4 Mosaic 8	
Medical history of GH therapy	GH (+) 3 GH (-) 9 others 4	GH (+) 27 GH (-) 23 Others 22	GH (+) 6 GH (-) 5 Others 1	
Age at initiating ERT (years)	22.3 ± 6.2 (16–37)	20.2 ± 6.5 (14–44)	13.0 ± 1.0 (12–14) (Age at menarche)	
Duration of ERT (years)	5.1 ± 3.3 (1–9)	11.2 ± 7.1 (5–32)		

Data are expressed as mean $\pm$ SD (range). *P* values were determined by Kruskal-Wallis test. NS, not statistically significant.

Fig. 1 Uterine length at first visit (1A) and latest visit (1B) in the PA group and the MC group. *P* values were determined by Kruskal-Wallis test. NS, not statistically significant.

Fig. 2 BMD at first visit (2A) and latest visit (2B) in the PA group and the MC group. *P* values were determined by Kruskal-Wallis test. NS, not statistically significant.

There were 37 patients in the PA group and 10 patients in the MC group. The PA group was divided into 2 groups; the late initiation group (21 patients) in which ERT was initiated at 18 years of age or older and the early initiation group (16 patients) in which ERT started before 18 years. The mean age of initiating ERT was 16.6 ± 1.2 years in the early initiation group and 19.9 ± 1.6 years in the late initiation group. The mean age of menarche in the MC group was 13 years. There was no significant difference in the duration of treatment between the late initiation group and the early initiation group (Table 2).

At the time of the latest visit, there were no significant differences between the ULs of patients in the early initiation group, the late initiation group, and the MC group (Fig. 3A).

The BMD of patients in the late initiation group at the latest visit (0.770 ± 0.107 ; $n = 16$) was significantly lower than that of patients in the early initiation group (0.858 ± 0.119 g/cm^2 ; $n = 21$) or the MC group (0.941 ± 0.118 g/cm^2 ; $n = 10$) (Fig. 3B). No significant difference in BMD was seen between the early initiation group and the MC group.

Table 2 Characteristics of the patients referred from KCMC and comparison of various values between PA group (Late and Early Initiation ERT) and MC group

	Primary amenorrhea (n = 37)		Menstrual cycle (n = 10)	P value
	Late initiation (n = 21)	Early initiation (n = 16)		
Age (years)	35.5 ± 8.2 (26 – 58)	28.8 ± 6.1 (18 – 40)	28.7 ± 6.4 (18 – 40)	NS ^(a)
Age at initial evaluation (years)	26.5 ± 7.7 (26 – 58)	23.6 ± 3.4 (17 – 29)	22.2 ± 4.7 (18 – 31)	NS ^(a)
Height (cm)	144.7 ± 5.3 (132.0 – 151.0)	146.2 ± 5.1 (134.0 – 151.0)	138.8 ± 5.5 (130.0 – 148.0)	0.012 ^(a)
BMI (kg/m ²)	21.6 ± 4.5 (15.0 – 33.6)	22.2 ± 3.1 (36.0 – 57.0)	23.9 ± 3.3 (19.0 – 29.0)	NS ^(a)
Karyotype	Monosomy 4 Structural abnormality 4 Mosaic 12 Unknown 1	Monosomy 4 Mosaic 11	Structural abnormality 4 Mosaic 6	
Medical history of GH therapy	GH (+) 11 GH (-) 4 others 6	GH (+) 8 GH (-) 4 others 4	GH (+) 5 GH (-) 4 others 1	
Age at initiating ERT (years)	19.9 ± 1.6 (18 – 22)	16.6 ± 1.2 (14 – 17)	12.5 ± 1.1 (11 – 14) (Age at menarche)	
Duration of ERT (years)	13.5 ± 5.7 (7 – 27)	12.8 ± 6.1 (1 – 23)		NS ^(b)

Data are expressed as mean ± SD (range). *P* values were determined by Kruskal-Wallis test for three groups, the late initiation, early initiation, and MC groups (a), while the Mann-Whitney test was used to compare two groups, the late initiation and early initiation groups (b). NS, not statistically significant.

Fig. 3 Comparison between early initiation and late initiation of ERT in uterine length (3A) and BMD (3B) at latest visit in the patients referred from KCMC.

P values were determined by Kruskal-Wallis test. NS, not statistically significant.

Discussion

Although several reports have mentioned the efficacy of ERT in increasing UL or BMD [4, 5, 6] few studies have addressed its effects on both uterine growth and bone maturation in a single study, in adolescents with TS [7, 8]. Our results demonstrate that ERT is effective in increasing both UL and BMD in adult TS patients with PA.

In this study, the increase in UL did not depend on

the age of commencing ERT. Significant differences in UL between patients with previous ERT and those with MC, and between patients without ERT and those with MC, all disappeared after ERT. This suggests that uterine growth can be achieved by ERT regardless of initiation age. This finding is consistent with previous studies showing that uterine size was insufficiently increased in adolescents [9–12] but could be improved in adults [13–15].

On the other hand, early initiation of ERT was nec-

essary for acquisition of bone mass. Women with TS require ERT for acquisition of peak bone mass and prevention of osteoporosis and bone fracture [16-18]. In this study, significant differences in BMD between patients who had previously received ERT and those without ERT disappeared once all had been treated with ERT. However, BMD in the PA group did not reach the level observed in the MC group since a significant difference in BMD remained between the patients with PA after receiving ERT and those with MC. These data showed that our ERT protocol was still inadequate to increase bone density.

However, it could be argued that early initiation of ERT is more effective. Kodama *et al.* previously demonstrated that estrogen therapy might be more effective if initiated by age 18 [19]. Similar results were obtained from the analysis of referred patients from KCMC; patients in the early initiation group were able to achieve a bone density equivalent to that in the MC group in which ERT was begun by the age of 18. Previous reports demonstrated the importance of adolescent age in terms of acquisition of bone mass. Theintz *et al.* reported that the bone growth rate is highest between 11 and 14 years of age [20] while Sabatie *et al.* reported that BMD increased dramatically between the ages of 10 and 14 [21]. Teegarden *et al.* demonstrated that young women attain 92% of their total body bone mineral content by 18 years of age [22]. If no signs of the onset of puberty are observed during adolescence, guidelines recommend starting ERT as early as 12 years of age [3].

The other factors that should be considered are GH therapy, exercise, food and nutrition. In this study, there was no significant difference among the groups in the history of GH treatment in any analysis. Since TS patients tend to have less active characteristics, [23] we recommend exercises to TS patients. Vitamin D deficiency is also observed in TS patients [24]. We did not measure 25-hydroxyvitamin D in our study mainly because 25-hydroxyvitamin D is not covered by medical insurance in Japan.

No clear direct correlation between uterine length and BMD increase was observed. There was a dissociation between the uterine length and any increase in BMD since other factors such as vitamin D are involved in the acquisition of bone mass.

There are some limitations to this study. First, the sample size is relatively small because of the setting in a referral center. Secondly, since this was a retrospective cohort study, the doses and types of drugs used for ERT in the original facilities are not uniform except in the KCMC and our hospital. Thirdly, adults accounted for a large proportion of the subjects and the current recommendation, in which ERT is introduced at a younger age, was not investigated in this study.

Nevertheless, the results of the present study are considered to be useful from a clinical point of view. Although it has been reported that the BMD of women with TS tends to be underestimated because of their short stature [25, 26], it has been found that they have sufficient bone mass, similar to those in the MC group in which height is significantly lower. In addition, by using BMD of the MC group as a positive control, it was possible to compare the BMD of the amenorrhea group.

In conclusion, ERT is effective in inducing both uterine growth and bone mass acquisition. However, early initiation of ERT would be necessary for acquisition of bone mass under these circumstances.

Further study is needed in order to establish an adequate regimen of ERT for TS patients.

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Disclosure

None of the authors has any potential conflict of interest associated with this research.

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