

Eldecalcitol increases bone mass in patients with Turner syndrome who have insufficient bone mass acquisition after estrogen replacement therapy

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Abstract. Most patients with Turner syndrome (TS) exhibit amenorrhea due to premature ovarian failure. Therefore, estrogen replacement therapy (ERT) is required; however, even after undergoing ERT, it is not rare for bone mass acquisition to be insufficient. This study was conducted in two stages, involving a cross-sectional and a prospective interventional study. We recruited 52 TS patients undergoing ERT due to amenorrhea (categorized into low (LB group; $n = 23$), and normal (NB group; $n = 29$) bone mass groups) and 7 TS patients who maintained ovarian function (spontaneous menstrual cycle group (MC group)) as controls. We compared bone associated markers between the three groups (LB, NB, and MC). Furthermore, the LB group had concomitant treatment with eldecalcitol (ELD) and ERT for 12 months. The bone mineral density (BMD) of the lumbar spine (L2-4) and the bone metabolism markers were then compared before and after the treatment. The bone metabolism markers were significantly higher in the LB group than the NB and MC groups. Furthermore, with the concomitant use of ELD and ERT in the LB group, BMD increased significantly (pre-treatment 0.710 ± 0.056 g/cm² vs. 0.736 ± 0.062 g/cm² after 12 months; $p < 0.001$). TS patients with insufficient bone mass acquisition even after ERT were characterized by a higher turnover in bone metabolism. Therefore, the concomitant use of ELD was considered an effective adjuvant therapy for increasing bone mass.

Key words: Turner syndrome, Osteoporosis, Bone metabolism, Bone mass, Eldecalcitol

TURNER SYNDROME (TS) is a condition caused by an X chromosome abnormality affecting approximately 1/2,500 females at birth. Most cases exhibit amenorrhea due to premature ovarian failure, and are at a high risk for osteoporosis [1]. As a result, estrogen replacement therapy (ERT) is needed not only for sexual maturation, but also for maintaining health and gaining bone mass. However, even after undergoing ERT, it is not rare for bone mass acquisition to be insufficient.

In previous studies, insufficient bone mass has been reported in patients with TS even when ERT was administered [2-4]; on the other hand, there have also been reports of sufficient bone mass being attained [5, 6]. Thus, findings from the evaluation of bone mass in

patients with TS are controversial. Reports to date mostly involve comparison with age-matched healthy females. However, physique and genetic background differences affect the evaluation of bone mass. Hence, these subjects cannot be considered appropriate for comparison. Some patients with TS have normal ovarian function and menstruate naturally [7]. Thus, the present analysis overcame the aforementioned issues by using patients with TS and normal ovarian function as positive controls for comparing bone mass and bone metabolism.

In addition, it is necessary to determine effective adjuvant therapies for patients with insufficient bone mass acquisition on ERT. However, no efficacious methods have been established to date. Eldecalcitol (ELD), an active vitamin D analog, increases bone mass and improves bone metabolism [8, 9]. Although it is also conceivably effective for patients with TS, there is no clinical data investigating the effect of ELD in TS. The aim of the present study was to identify the characteristics of bone metabolism and low bone mass in patients

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with TS even after ERT, and to determine the effectiveness of ELD as an adjuvant therapy for bone mass acquisition.

Materials and Methods

Participants

This study was conducted from April 2013 to October 2015 at the Yokohama City University Hospital and the Yokohama City University Medical Center. Participants were TS patients followed-up at the women's health outpatient clinic in our department. The ERT treatment at these institutions as a rule consists of conjugated equine estrogens (CEE; 0.625 mg/day for 21 days) and medroxyprogesterone acetate (MPA; 5 mg/day for 11 days), or transdermal estradiol 0.72 mg/patch for 22 days with MPA (5 mg/day for 11 days). To ensure that only patients who had undergone ERT for a sufficient time period and with stable bone density were studied, patients under the age of 20, those undergoing ERT at low doses, and those who had been undergoing ERT at an adult dose for less than two years, were excluded. Furthermore, since TS is known to have a variety of complications such as diabetes [10, 11], diabetic patients (with a treatment history or HbA1c $\geq 6.5\%$), chronic kidney disease patients (estimated glomerular filtration rate (eGFR) < 60 mg/min/BSAm²), and patients undergoing steroid treatment due to secondary osteoporosis were excluded. Those who were already using ELD were also excluded. The remaining 59 patients with TS were enrolled.

Bone mineral measurement

Bone density was evaluated using the dual-energy X-ray absorptiometry method on a QDR 2000/W device (Hologic, Waltham, MA, USA) with the areal bone mineral density (BMD) of the lumbar spine (L2-4) as the indicator. The 2001 Osteoporosis Diagnostic Criteria of the Japanese Society for Bone and Mineral Research were used as the standards for osteopenia and osteoporosis [12]. With the young adult mean (YAM) as a benchmark, osteopenia was defined as $\text{YAM} < 80\%$ (-1.63 standard deviation (SD)) and osteoporosis as $\text{YAM} < 70\%$ (-2.45 SD).

Biochemical markers associated with bone metabolism

Bone-associated markers were measured as follows. We evaluated urinary cross-linked N-telopeptides of type I collagen (uNTX) as a bone absorption marker, serum

intact procollagen type I N-terminal propeptide (intact-P1NP) as a bone formation marker, urinary pentosidine and serum homocysteine as bone quality markers, serum 25-hydroxyvitamin D (25(OH)D) as a vitamin D sufficiency indicator, and undercarboxylated osteocalcin (ucOC) as a vitamin K sufficiency indicator. Japan cut of levels for 25(OH)D were used as vitamin D deficient (< 20 ng/mL) and vitamin D insufficient (> 20 ng/mL and < 30 ng/mL) [13]. Pentosidine testing *via* a high-performance liquid chromatography method was consigned to LSI Medience Corp, Tokyo, Japan. For 25(OH)D measurement, an automated chemiluminescence measurement method (measurement reagent: Liaison 25-hydroxyvitamin D Total (DiaSorin Inc., Stillwater, MN, USA)) was used at Kyowa Medex Co., Ltd., Shizuoka, Japan.

Study design

The study was conducted in two stages, involving a cross-sectional and a prospective interventional study (Fig. 1).

Study 1: Cross-sectional: comparison between amenorrhea (low and normal bone mass) and spontaneous menstrual cycle groups

Of the 59 patients with TS, 52 required ERT due to amenorrhea (categorized into low (LB; $n = 23$), and normal (NB; $n = 29$) bone mass groups), and in 7 patients (spontaneous menstrual cycle group; MC group), ovarian function and menstruation were maintained. In the 23 LB bone mass patients, 11 had osteoporosis ($\text{YAM} < 70\%$) while 12 had osteopenia ($\text{YAM} < 80\%$) after ERT. No osteoporosis or osteopenia occurred among the seven patients with a spontaneous menstrual cycle. The MC group was used as the positive control, and bone-associated factors were compared between the LB, NB, and MC groups.

Study 2: Longitudinal comparison of bone mineral density and biochemical markers associated with bone metabolism before and after treatment with eldecacitol

We investigated the changes in bone mass and bone-associated factors when ELD and ERT were used concomitantly in the 23 LB patients. After the commencement of the concomitant ELD and ERT use, BMD was measured on the 6th and 12th months, and uNTX, intact-P1NP, and pentosidine were measured on the 3rd, 6th, and 12th months. Three patients who decided to discontinue outpatient care after the commencement of the intervention were excluded from the analysis; hence, the

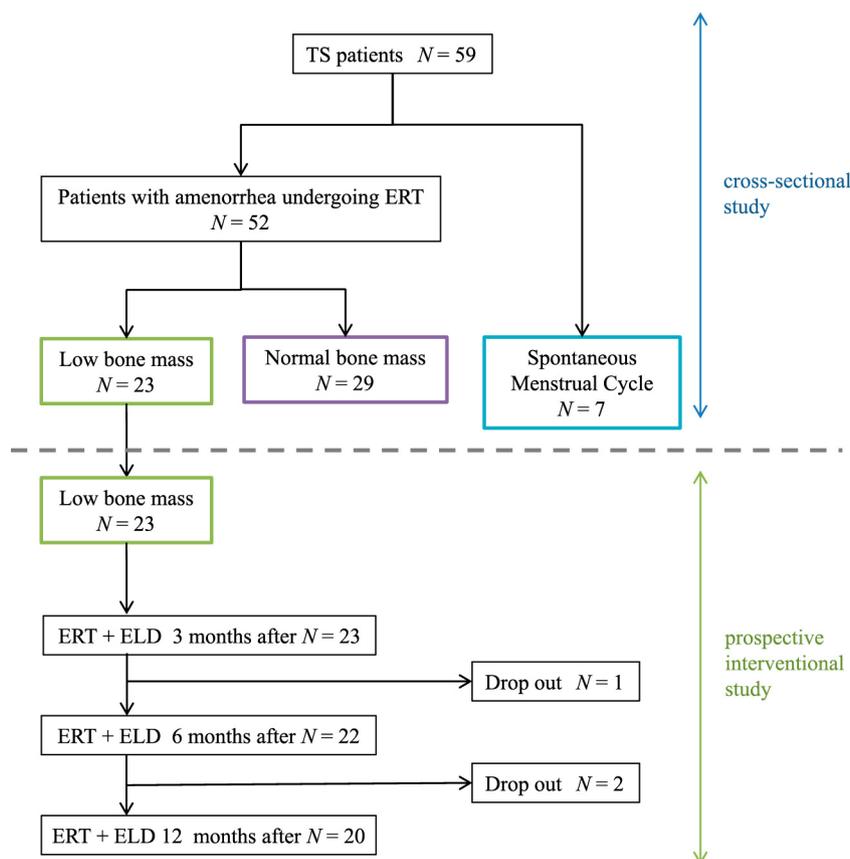


Fig. 1 Flow diagram of the study
 TS, Turner syndrome; ERT, estrogen replacement therapy; ELD, eldecalcitol

analysis was based on data from the remaining 20 LB patients. The ELD dose was 0.75 µg/day. Blood calcium (Ca) and urine Ca/creatinine (Cre) were regularly measured. The ELD dose was reduced to 0.5 µg/day if hypercalcemia (blood Ca > 11.5 mg/dL) or hypercalciuria (urine Ca/Cre > 0.4) was detected.

Ethical considerations

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committees of Yokohama City University School of Medicine (IRB no. Study 1; B130707012, Study 2; B130307013). All patients provided written informed consent. The trial was registered with the University Hospital Medical Information Network (UMIN) Center under the identifier UMIN no. Study 1; 000027948 and Study 2; 000027959.

Statistical analysis

Statistical analysis used SPSS 20.0 software (SPSS, Chicago, IL, USA). Patients' background characteristics

and results are reported as mean ± SD. In the cross-sectional study, to compare the three groups, Kruskal Wallis test was used, and statistically significant variables were compared between pairs of groups with the Mann-Whitney U test (Fisher's least significant difference post hoc test [14]). The χ² test was used for comparisons of percentages. In the prospective interventional study, Wilcoxon's signed rank test was used to evaluate the change before and after ELD intervention. The significance threshold was *p* < 0.05.

Results

Baseline characteristics

Table 1 shows patients' baseline characteristics. The BMD in the LB, NB, and MC groups were 0.716 ± 0.053, 0.909 ± 0.08, and 0.926 ± 0.054 g/cm², respectively (*p* < 0.001). The recent BMD annual rate of change was sufficiently low in all three groups, and thus the bone mass changes due to ERT were considered sta-

Table 1 Clinical characteristics of Turner syndrome patients

	Amenorrhea		Spontaneous Menstrual Cycle (C) (N = 7)	p-value			
	Low bone mass (A) (N = 23)	Normal bone mass (B) (N = 29)		A vs. B	B vs. C ^a	A vs. B ^b	A vs. C ^b
DXA (Lumbar spine)							
BMD (g/cm ²)	0.716 ± 0.053	0.909 ± 0.08	0.926 ± 0.054	<0.001*	<0.001*	<0.001*	0.223
YAM (%)	70.8 ± 5.3	89.9 ± 8.3	92.0 ± 7.5	<0.001*	<0.001*	<0.001*	0.288
T-score	-2.57 ± 0.47	-0.85 ± 0.70	-0.67 ± 0.68	<0.001*	<0.001*	<0.001*	0.399
Z-score	-2.59 ± 0.51	-1.20 ± 2.00	-0.69 ± 0.74	<0.001*	<0.001*	<0.001*	0.644
BMD % change/year (%)	-0.26 ± 1.3	+0.57 ± 1.1	-0.01 ± 1.2	0.238			
Age (years)	31.9 ± 6.1	32.5 ± 7.3	28.6 ± 8.1	0.345			
Height (cm)	146.1 ± 4.9	145.8 ± 6.4	139.4 ± 6.7	0.073			
Weight (kg)	43.2 ± 6.6	49.6 ± 9.3	46.3 ± 5.1	0.013*	0.004*	0.105	0.588
Body mass index (kg/m ²)	20.2 ± 2.2	23.4 ± 4.4	23.9 ± 3.2	0.003*	0.003*	0.012*	0.675
Karyotype							
45, XO	5 (21.7%)	9 (31.0%)	—				
Structural abnormality	5 (21.7%)	6 (20.7%)	2 (28.6%)	0.292 ^c			
Mosaic	13 (56.5%)	11 (37.9%)	5 (71.4%)				
Unknown	—	3 (10.3%)	—				
Age at initiating ERT (years)	19.9 ± 5.8	18.1 ± 3.0	—	0.274 ^b			
Duration of ERT (years)	12.1 ± 5.7	12.0 ± 6.3	—	0.399 ^b			
Serum Ca (mg/dL)	9.3 ± 0.3	9.4 ± 0.4	9.1 ± 0.3	0.167			
Route of ERT							
Oral	17 (73.9%)	23 (79.3%)	—				
Transdermal	6 (26.1%)	6 (20.7%)	—	0.746 ^c			
medical history of GH therapy							
GH(+)	12 (52.2%)	9 (31.0%)	3 (42.9%)				
GH(-)	11 (47.8%)	20 (69.0%)	4 (57.1%)	0.303 ^c			

Data are expressed as mean ± SD (range). *P* values were determined by Kruskal-Wallis test for three groups (a), when the Mann-Whitney test was used to compare between two groups (b), χ^2 -test (c). **P*-value less than 0.05 are considered statistically significant. DXA, dual energy X-ray absorptiometry; YAM, young adult mean; BMD, bone mineral density; ERT, estrogen replacement therapy; GH, growth hormone replacement therapy

ble. A significant difference also occurred with the body mass index (BMI) (20.2 ± 2.2 vs. 23.4 ± 4.4 vs. 23.9 ± 3.2 kg/m²; *p* = 0.003). There was no significant difference in age, height, chromosomal karyotype, or presence of growth hormone therapy between the three groups. In TS patients with amenorrhea, there was no difference between the LB and NB groups in the ERT commencement age, dose, route of administration, or length of administration.

Study 1: Cross-sectional: comparison between amenorrhea (low and normal bone mass) and spontaneous menstrual cycle groups

Table 2 shows the results of the bone-associated markers. Significant differences occurred between the three groups with uNTX, a bone absorption marker (44.8

± 17.7 vs. 36.0 ± 18.0 vs. 30.2 ± 9.8 nmol BCE/mmol Cr; *p* = 0.003) and intact-P1NP, a bone formation marker (63.5 ± 27.4 vs. 49.0 ± 21.4 vs. 35.4 ± 9.0 µg/mL; *p* = 0.012). However, no significant differences were found with pentosidine and homocysteine, markers of bone quality, although the pentosidine level in the LB group tended to be higher than in the NB and MC groups (35.4 ± 17.5 vs. 32.2 ± 11.1 vs. 24.7 ± 9.8 pmol/mgCr; *p* = 0.076). No significant difference was found in uOC between the three groups. Although 25(OH)D exhibited low values in all groups, no significant difference was found with 25(OH)D between the three groups (13.8 ± 5.4 vs. 12.9 ± 4.2 vs. 15.1 ± 4.4 ng/mL; *p* = 0.567). Vitamin D insufficiency (*i.e.* 25(OH)D < 30 ng/mL) was found in 100%, while vitamin D deficiency (*i.e.* 25(OH)D < 20 ng/mL) was found in 98.5% of all groups.

Table 2 Biochemical markers associated with bone metabolism in the Turner syndrome patients

	Amenorrhea		Spontaneous Menstrual Cycle (C) (N = 7)	p-value			
	Low bone mass (A) (N = 23)	Normal bone mass (B) (N = 29)		A vs. B	B vs. C ^a	A vs. C ^b	B vs. C ^b
uNTX (nmol BCE/mmol Cr)	44.8 ± 17.7	36.0 ± 18.0	30.2 ± 9.8	0.003*	0.004*	0.005*	0.470
Intact-P1NP (µg/mL)	63.5 ± 27.4	49.0 ± 21.4	35.4 ± 9.0	0.012*	0.049*	0.007*	0.101
Pentosidine (pmol/mgCr)	35.4 ± 17.5	32.2 ± 11.1	24.7 ± 9.8	0.076			
Homocysteine (nmol/mL)	8.1 ± 1.9	7.84 ± 1.72	8.37 ± 3.15	0.940			
25(OH)D (ng/mL)	13.8 ± 5.4	12.9 ± 4.2	15.1 ± 4.4	0.567			
ucOC (ng/mL)	5.34 ± 2.79	5.24 ± 3.55	3.60 ± 0.29	0.497			

Data are represented as mean ± SD. P values were determined by Kruskal-Wallis test for three groups (a), when the Mann-Whitney test was used to compare between two groups (b). *P-value less than 0.05 are considered statistically significant. uNTX, urinary cross-linked N-telopeptides of type I collagen; intact-P1NP, serum intact procollagen type I N-terminal propeptide; 25(OH)D, serum 25-hydroxyvitamin D; ucOC, undercarboxylated osteocalcin

Table 3 Changes in bone mineral density and biochemical markers associated with the bone metabolism before and after treatment with eldecalcitol

	before	3 months after	6 months after	12 months after
DXA (Lumbar spine)				
BMD (g/cm ²)	0.710 ± 0.056	—	0.731 ± 0.07*	0.736 ± 0.062*
T-score	-2.61 ± 0.49	—	-2.45 ± 0.58*	-2.41 ± 0.54*
Z-score	-2.62 ± 0.55	—	-2.46 ± 0.63*	-2.37 ± 0.57*
uNTX (nmol BCE/mmol Cr)	49.7 ± 20.7	43.6 ± 18.7	46.7 ± 23.8	46.2 ± 26.1
Intact-P1NP (µg/mL)	63.9 ± 28.6	53.2 ± 28.0*	51.9 ± 27.8*	47.7 ± 24.7*
Pentosidine (pmol/mgCr)	38.7 ± 19.7	31.0 ± 11.9	29.6 ± 7.5*	27.0 ± 10.7*

Data are represented as mean ± SD. P values were determined Wilcoxon signed-rank test for comparison before and after treatment with eldecalcitol. P-value less than 0.05 are considered statistically significant. *p < 0.05 versus before treatment with eldecalcitol. DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density; uNTX, urinary cross-linked N-telopeptides of type I collagen; intact-P1NP, serum intact procollagen type I N-terminal propeptide

Next, BMD, BMI, uNTX, and intact-P1NP, which showed significant differences among the three groups, were compared among pairs of groups (Tables 1 & 2). BMD (g/cm²) was significantly lower in the LB group (0.716 ± 0.053) than the NB (0.909 ± 0.08; p < 0.001) and MC (0.926 ± 0.054; p < 0.001) groups. BMI (kg/m²) was significantly lower in the LB group (20.2 ± 2.2) than the NB (23.4 ± 4.4; p = 0.003) and MC (23.9 ± 3.2; p = 0.012) groups. uNTX (nmol BCE/mmol Cr) was significantly higher in the LB group (44.8 ± 17.7) than the NB (36.0 ± 18.0; p = 0.004) and MC (30.2 ± 9.8; p = 0.005) groups. Intact-P1NP (µg/mL) was also significantly higher in the LB group (63.5 ± 27.4) than the NB (49.0 ± 21.4; p = 0.049) and MC (35.4 ± 9.0; p = 0.007) groups. However, no significant difference was found between the NB and MC groups for BMD, BMI, uNTX, or intact-P1NP.

Study 2: Longitudinal comparison of bone mineral density and biochemical markers associated with bone metabolism before and after treatment with eldecalcitol

Table 3 shows the changes in BMD and bone-associated markers with concomitant ELD and ERT use. BMD increased significantly (pre-treatment 0.710 ± 0.056 vs. 0.736 ± 0.062 g/cm² after 12 months; p < 0.001). With intact-P1NP (pre-treatment 63.9 ± 28.6 vs. 47.7 ± 24.7 µg/mL after 12 months; p = 0.002) and pentosidine (pre-treatment 38.7 ± 19.7 vs. 27.0 ± 10.7 pmol/mgCr after 12 months; p = 0.004), significant decrease occurred. No significant change was seen in uNTX.

Fig. 2 shows the rate of change in the bone-associated markers. The rate of change of BMD before ELD to 12 months after treatment was +3.7 ± 2.8% (Fig. 2A). uNTX change (by -3.7 ± 42.3% after 12 months) was

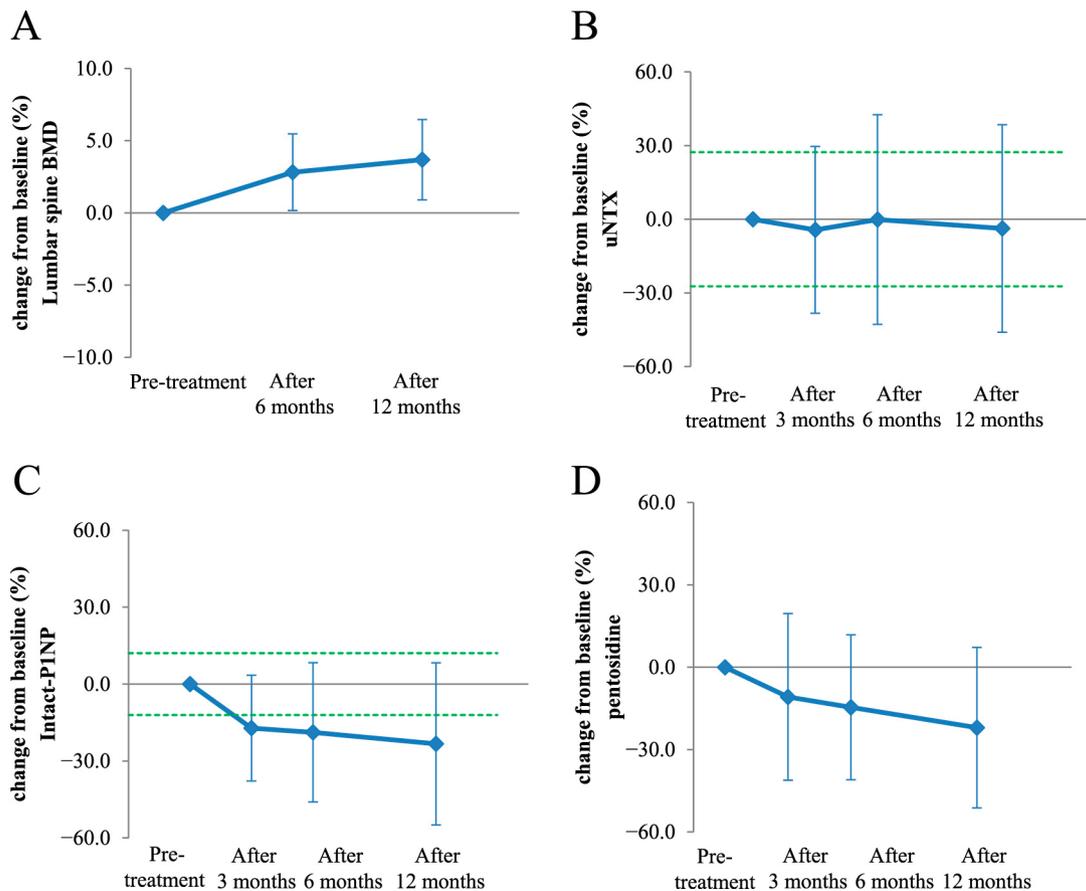


Fig. 2 Percent changes of the levels of lumbar spine BMD (Fig. 2A), uNTX (Fig. 2B), intact-P1NP (Fig. 2C), and pentosidine (Fig. 2D) from the baseline

Data are represented as mean \pm SD. *P* values were determined Wilcoxon signed-rank test for comparison before and after treatment with eldecalcitol. *P*-value less than 0.05 are considered statistically significant. **p* < 0.05 versus before treatment with eldecalcitol. BMD, bone mineral density; uNTX, Urinary cross-linked N-telopeptides of type I collagen; Intact-P1NP serum intact procollagen type I N-terminal propeptide

within the threshold of minimum significant change of 27.3% (Fig. 2B), while intact-P1NP change (by $-23.3 \pm 31.6\%$ after 12 months) exceeded the minimum significant change of 12.1% (Fig. 2C). Pentosidine changed by $-22.0 \pm 29.2\%$ after 12 months (Fig. 2D).

Discussion

Our findings in this study were as follows. First, the LB group exhibited a significantly higher rate of bone metabolism than the NB and the MC groups. Second, 25(OH)D exhibited a low value in all the TS groups. Third, when ELD was concomitantly used with ERT in the LB group, bone mass increased significantly while intact-P1NP and pentosidine decreased significantly.

In previous reports, patients with TS exhibited low

values in the measurements of areal BMD using the dual-energy X-ray absorptiometry method [2-4]. This is because low body height, characteristic of patients with TS, led to an underestimation of areal BMD. It has been reported that volumetric BMD (a volumetric bone parameter correcting for physique), was not different in TS patients compared to healthy women [5, 6], however, areal BMD is a useful evaluation method used universally for osteoporosis. In this study, TS patients with spontaneous menstrual cycle were used as positive controls to compare the areal BMD values against those of TS patients with amenorrhea who underwent ERT. TS patients with insufficient bone mass acquisition and significantly lower areal BMD compared to the MC group despite being the same in age, height, and genetic background are believed to certainly exist. In a prior study,

the areal BMD of TS patients with spontaneous menstrual cycles were reported as 0.937–0.945 g/cm² [15], a similar result to the present study finding. In this study, there was no difference between the LB and NB groups in ERT administration method, dose, commencement time, and treatment period. The fact that TS patients with low bone mass were comparatively slim and had a higher turnover of bone metabolism is an important consideration for adjuvant therapies.

Our study was the first to evaluate 25(OH)D in patients with TS in Japan. 25(OH)D exhibited a low value in all groups. No significant difference was found between the three groups; hence, it does not contribute as a factor to bone mass acquisition with ERT. However, it is an important factor for improving bone strength. Prior studies have reported 25(OH)D values in TS patients with approximately 23.5–33.6 ng/mL [5, 16], lower than values in healthy women. In this study, the values in TS patients were even lower than in prior studies. The 25(OH)D values in Japanese population overall (approximately 16.7–18.1 ng/mL [17, 18], and 18.7–21.2 ng/mL [19, 20] in youths), are reportedly lower than those generally reported overseas. The fact that most of the patients in this study were Vitamin D deficient is considered a characteristic of TS as well as a racial/ethnic effect in the Japanese population. In addition, the LB group had a significantly lower BMI. As a result, vitamin D supplementation as well as nutritional management with the goal of an appropriate body weight may be effective for maintaining bone mass.

Due to the above-mentioned factors, correcting bone metabolic turnover and vitamin D supplementation are conceivably effective treatment methods for TS patients with low bone mass even after ERT. ELD is a drug that is superior in its bone mass increasing effect and bone metabolism improving effect compared to the pre-existing active vitamin D analog (alfacalcidol) [8, 21]. It was reported that combining the use of alfacalcidol led to a greater improvement in bone density than ERT alone [22], but it is expected that the effect from using ELD with ERT is even greater still. In a prior study of postmenopausal osteoporosis patients, ELD increased lumbar BMD by 2.3% after 12 months. In this study as well, lumbar BMD increased by $+3.7 \pm 2.8\%$ at the 12-month mark, which was the expected result. ELD can be considered a highly effective adjuvant therapy for TS patients in whom ERT bone mass increasing effect was considered stable.

The concomitant use of ELD led to a decrease in

PINP, a bone formation marker, while uNTx, a bone absorption marker, was not suppressed. We had expected that bone metabolism would be suppressed as shown in previous studies, but there were divergent changes in uNTx and PINP. We believe that the reasons for the divergent changes in uNTx and PINP were as follows. First, in a previous study, it was shown that ELD normalizes, but does not overly suppress the bone turnover regardless of the baseline levels of bone turnover markers [21]. More specifically, ELD is said to suppress bone absorption when the turnover of bone metabolism is considerably high but does not overly suppresses it when the turnover is not so high. Additionally, the participants were limited to those who had been administered with ERT for a sufficient amount of time and had showed stable levels of BMD. We assume that, because of this, bone metabolism was regulated to an appropriate state by ELD intervention, while uNTx and PINP were suppressed to a certain degree by ERT. In a previous study that examined the efficacy of ELD, cases of untreated postmenopausal osteoporosis were studied, and the uNTx level before ELD intervention was 58.1 nmol BCE/mol Cr [21]. Compared to this, the uNTx level reported in the present study was low at 44.8 nmol BCE/mol Cr. This may be the reason why ELD intervention caused little change. In addition, a comparison between uNTx and PINP showed uNTx to have had more diurnal as well as day-to-day variations. While PINP showed significant decrease, this might have biased our assessment about the changes in uNTx. Unfortunately, we were unable to elucidate a reasonable mechanism to explain “why the BMD increased after ELD”, in the present study. ELD is a feasible treatment, and we believe that the concomitant use of ELD under the premise that there is a vitamin D deficiency is an effective treatment consideration for Turner syndrome patients with insufficient bone mass. In the present study, the subjects were limited to those that had been administered ERT for a sufficient amount of time and showed stable levels of BMD.

Bone density and bone quality are both important for bone strength. Pentosidine has been reported as useful for evaluating bone quality [23, 24]. Pentosidine is one of the advanced glycation end products of bone collagen, and its accumulation signifies bone quality degradation. One of the reasons for the elevated pentosidine is the oxidative stress accompanying low estrogen levels. In this study, there was a tendency for the highest pentosidine levels to be found in the LB group, followed by the

NB and the MC groups, although no significant difference was found between the three groups. The participants in our study were young, and it is conceivable that a certain degree of bone quality was maintained through ERT, but it is also possible that a significant difference would be found in this result if we had a larger study population. On the other hand, pentosidine levels decreased significantly in the LB group upon concomitant use of ELD with ERT. It is possible that bone quality improved. ELD has been reported to have a bone mass increasing effect and a bone quality improving effect [25, 26]. In particular, Saito *et al.* reported in a study using monkeys that pentosidine decreased significantly and bone strength increased upon ELD use [25].

When considering adjuvant therapy for TS patients with insufficient bone mass acquisition through ERT, bisphosphonate is effective; however, its use is concerning, due to its tendency to accumulate and its effect on subsequent pregnancies. Additionally, its long-term safety is of concern due to bone quality degradation caused by excessive bone metabolism suppression. In fact, elevated pentosidine due to the long-term bisphosphonate use has been reported [27, 28]. However, ELD is an active vitamin D analog believed to be highly safe in youths due to its pharmacological effect and lack of accumulation. Furthermore, it is an agent expected to improve bone quality. In this study, concomitant ELD use significantly increased bone mass, but no excessive suppression of bone metabolism was seen, and pentosidine also decreased significantly. This suggests its use as a potential adjuvant therapy effective for both bone mass and bone quality in patients with TS and with insufficient bone mass from ERT alone.

This study has some limitations. First, the initial timing and dose of ERT were reported to be important for increasing bone mass [29], but participants in this study had a late average ERT start age of 19.9 ± 5.8 years. In addition, the ERT dose used in Japan is lower than that used abroad. Differences in ovarian function before ERT may be a contributory factor to the differences in BMD that were seen in the present study. Most of the participants continued on ERT after being referred from the pediatric departments of various institutions to our hospital, indicating that they had been on ERT before their

first visit to our hospital. Unfortunately, we do not have the data from the laboratory tests performed before the initiation of ERT. Second, the study on the effectiveness of concomitant eldcalcitol use with ERT had no control group. In addition, the number of patients was low, and an effect of selection bias and confounding factors cannot be ruled out. In order to observe the effectiveness of eldcalcitol, further investigation through a randomized, controlled trial is needed.

This study is novel in the following ways. First, publications available to date often involve a comparison with age-matched, healthy women. In contrast, this study used TS patients with normal ovarian function as positive controls, thus overcoming the problem of differences in physical condition and genetic backgrounds. Second, we elucidated vitamin D deficiency in TS patients in Japan. Third, we evaluated bone quality in these TS patients.

Conclusion

The characteristics of TS patients with insufficient bone mass acquisition even when ERT was administered due to premature ovarian failure are a low BMI and a high turnover of bone metabolism. In addition, many patients with TS are vitamin D deficient, and concomitant use of ELD in such patients is considered an effective adjuvant therapy for increasing bone mass.

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Disclosure

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

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