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Bone Mineral Density in Adult Patients with Turner's Syndrome: Analyses of the Effectiveness of GH and Ovarian Steroid Hormone Replacement Therapies

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Abstract. To analyze the effects of treatments with GH and cyclic estrogen/progesterone (E/P) replacement on bone mineralization in patients with Turner's syndrome (TS), bone mineral density (BMD) was measured longitudinally. BMDs of the whole body and the lumbar spine in 16 adult female patients with TS (17–38 year old; 0–20 years by length of E/P treatment) were assessed using dual energy X-ray absorptiometry one to 5 times over a treatment period of up to 7 years maximum. GH treatment was performed in 9 cases (GH group), but not in the remaining 7 (non-GH group). E/P replacement therapy was initiated in all patients after they finished GH administration. The BMDs of both the whole body and the lumbar spine in the patients with TS were significantly less than those in age-matched normal subjects, and did not improve with E/P treatment. Although there were no differences in final body height and age at the beginning of E/P administration between the GH and non-GH groups, whole body BMD in the GH group was significantly lower than that in the non-GH group. These results indicate that GH administration in childhood and adolescence and E/P treatment in adulthood did not increase bone mineralization in the TS patients. Therefore, we can conclude that the optimal protocol of hormonal replacement therapy with GH and E/P during childhood and adolescence should be established as soon as possible.

Key words: Turner's syndrome, Bone mineral density, GH therapy, Estrogen/progesterone
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TURNER'S syndrome (TS) is a common chromosomal abnormality that is characterized by the presence of only one functioning X chromosome, the other being missing or abnormal. Most girls with TS are diagnosed in childhood by karyotype examination after recognition of the characteristic features of the syndrome, namely, short stature, stunted pubertal development and primary amenorrhea [1, 2]. Additionally, osteoporosis has been listed as an associated feature of TS patients [3–7]. Hence the management of adult TS patients requires a focus on both assisting sexual maturation and preventing osteoporosis.

Recently, GH therapy using recombinant human GH has been shown to improve the growth velocity of girls with TS in childhood, which increases their final height [8]. To induce and maintain secondary sexual

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maturation, estrogen with progesterone is administered. This cyclic estrogen/progesterone (E/P) therapy is usually performed continuously once GH therapy has stopped and/or sufficient stature has been obtained. On the other hand, there is no therapeutic agreement regarding how best to prevent osteoporosis. Although a number of reports concerning the bone mineralization of TS subjects are available, the effects of various treatments containing GH and E/P therapies remain controversial [9] for a variety of reasons: the studies involved only a short follow-up period, the patients concerned were young in age (not adult), and the population analyzed was small.

In the present study, we measured bone mineral density (BMD) in TS patients at over 17 years of age who received E/P therapy with or without GH administration in childhood and/or in adolescence. The effects of GH and E/P treatments on BMD in those patients were examined in a longitudinal manner.

Materials and Methods

Patients

Sixteen adult Japanese women with TS participated in the study. The mean age of the patients was 29.1 ± 5.2 years (range 19–39 years) when final BMD analyses were performed. In 5 patients, the classical 45, X karyotype was present, while the other patients had various mosaicisms and X isochromosomes (Table 1).

Nine TS patients had been treated with recombinant GH (GH group) from 10–19 years old for 10 to 122 months. In our hospitals, GH therapy began when growth failure became apparent as shorter stature than –2 standard deviations (SDs) and/or slower growth ve-

Table 1. Patient profiles with clinical data during GH and E/P therapies.

Case No.	Karyotype	GH therapy started		Duration of GH therapy	at E/P therapy started						Duration of E/P therapy
		Age (y.o.)	Height (cm)	(mos)	Age (y.o.)	Height (cm)	Weight (kg)	BMI	LH (mIU/ml)	FSH (mIU/ml)	(mos)
GH group											
1	45, X	12	122	99	23	141	43	21.6	22	65	16
2	46, X, +mar	19	145	10	20	145	42	20.0	ND	ND	152
3	46, X, i(Xq)	12	121	84	19	147	39	18.0	20	110	21
4	46, X, i(Xp)	14	130	89	22	140	51	26.0	26	73	47
5	45, X/46, XX	10	104	122	23	136	43	23.2	41	82	0
6	45, X/46, XY	11	120	77	17	150	47	20.9	40	70	26
7	45, X/46, X, +mar	12	130	97	20	140	39	19.9	28	65	72
8	45, X/46, X, +mar	16	128	44	20	135	46	25.2	ND	ND	110
9	45, X/46, Xi(Xq)	13	128	70	20	140	46	23.5	32	55	75
	Mean ± SD	13.2 ± 2.8	125.3 ± 10.9	77 ± 33	20.4 ± 1.9	141.6 ± 4.9	44.0 ± 3.9	21.6 ± 2.6			58 ± 50
Non-GH group											
1	45, X				17	143	48	23.5	ND	ND	163
2	45, X				17	136	43	23.3	ND	ND	238
3	45, X				18	139	48	24.8	ND	ND	157
4	45, X				24	142	45	22.3	27	95	102
5	46, X, i(Xq)				20	146	48	22.5	30	62	76
6	46, X, del(X)(q21)				23	157	50	20.3	42	78	12
7	45, X/46, X, r(Y)/46, X, dirc(Y)/46, X, terc(Y)				23	146	49	23.0	29	81	25
	Mean ± SD				20.3 ± 3.0	144.1 ± 6.7	47.3 ± 2.4	22.8 ± 1.4			110 ± 81

y.o.: years old, mos: months, BMI: body mass index, ND: not done

locity than −1.5 SDs for more than two years from the mean of girls of matching age. GH therapy continued until growth was completed. The other 7 TS patients did not receive GH therapy (non-GH group). Three patients in the non-GH group (Case Nos. 5–7 in Table 1) were out of the GH-therapy indication mentioned above. Human GH was not available for clinical use when three of the patients (Case Nos. 1–3) were diagnosed as TS, and the other one patient (Case No. 4) had not visited a hospital until 24 years of age after first getting diagnosed as TS at 12 years old. None of the women received treatment with estrogen prior to completion of GH therapy and none were treated with anabolic steroids.

At the time of E/P therapy started, body height and weight in the TS patients were 142.7 ± 5.7 cm (range 135–157 cm) and 45.4 ± 3.7 kg (range 39–51 kg), respectively. None of the TS women in the present study had spontaneous menstrual cycles, and all had delayed secondary sexual development. Hormone analyses indicated hypergonadotropic hypogonadism. Serum estradiol levels were less than 10 pg/ml. Serum FSH and LH concentrations are indicated in Table 1.

E/P replacement began at the age of 20.4 ± 2.4 years (range 17–24 years), using an E/P preparation containing mestranol (0.04–0.08 mg/day, Devocin, Shionogi Pharmaceutical Co., Tokyo, Japan), which was administered for 20–21 days and chlormadinone acetate (4–6 mg/day, Lutoral, Shionogi Pharmaceutical Co., Japan), which was given along with mestranol for the last 7–10 days of a cycle. This cyclic E/P therapy was performed for 81 ± 68 months (range 0–238 months). Following E/P administration, uterine bleeding was observed in all patients, and E/P therapy has been ongoing.

Measurement of BMD

BMDs (g/cm^2) of whole body and lumbar spine (L1-L4) in the TS patients were measured by dual energy X-ray absorptiometry (DXA) using a Hologic QDR 1000 scanner. BMD measurements were performed once to 5 times at 1–2 year intervals during a maximum time period of 7 years. All the procedures were performed after taking informed consent from the patients.

As a control, whole body and lumbar spine BMDs were measured in about 100 women of equivalent age at Nagoya University Hospital. The mean ± 2 SDs was used for normal range.

Data analysis

Unpaired Student's *t*-test was used for comparisons between TS patients and control populations or between GH and non-GH groups. Means were expressed as SD, and significance was defined as $P<0.05$.

Results

Longitudinal Change of BMD in TS Patients

Changes in BMD in the whole body (Fig. 1-A) and lumber spine (Fig. 1-B) in the 16 individual patients with TS were 0.72–1.09 g/cm^2 and 0.71–1.03 g/cm^2, respectively. There were various patterns of BMD changes through the follow-up period; some cases showed an increase in BMD, while others did not. The BMD values in the TS patients in any timeperiod were less than the mean BMDs of age-related controls, except in one case.

To analyze the effects of E/P therapy on BMD, BMD changes in the whole body and lumbar spine (Fig. 1-C) in the TS patients were evaluated longitudinally. No clear correlation between the length of E/P treatment and BMD values was seen. Further analysis of each TS patient data set was performed, using the mean BMD levels of their whole body and lumbar spine.

Comparison of BMD in TS Patients with Normal Controls

BMDs of the whole body and lumbar spine in the TS patients were 0.92 ± 0.09 g/cm^2 and 0.81 ± 0.09 g/cm^2, respectively, significantly less than BMD values in normal subjects (1.10 ± 0.09 g/cm^2 in whole body and 1.05 ± 0.10 g/cm^2 in lumbar spine).

Differences in BMD between GH and Non-GH Groups

To analyze the relationship between bone mineralization and GH therapy throughout childhood and adolescence, various parameters in the GH group were compared with those in the non-GH group (Tables 1 and 2). There were no significant differences in final height between the GH and non-GH groups (Table 1).

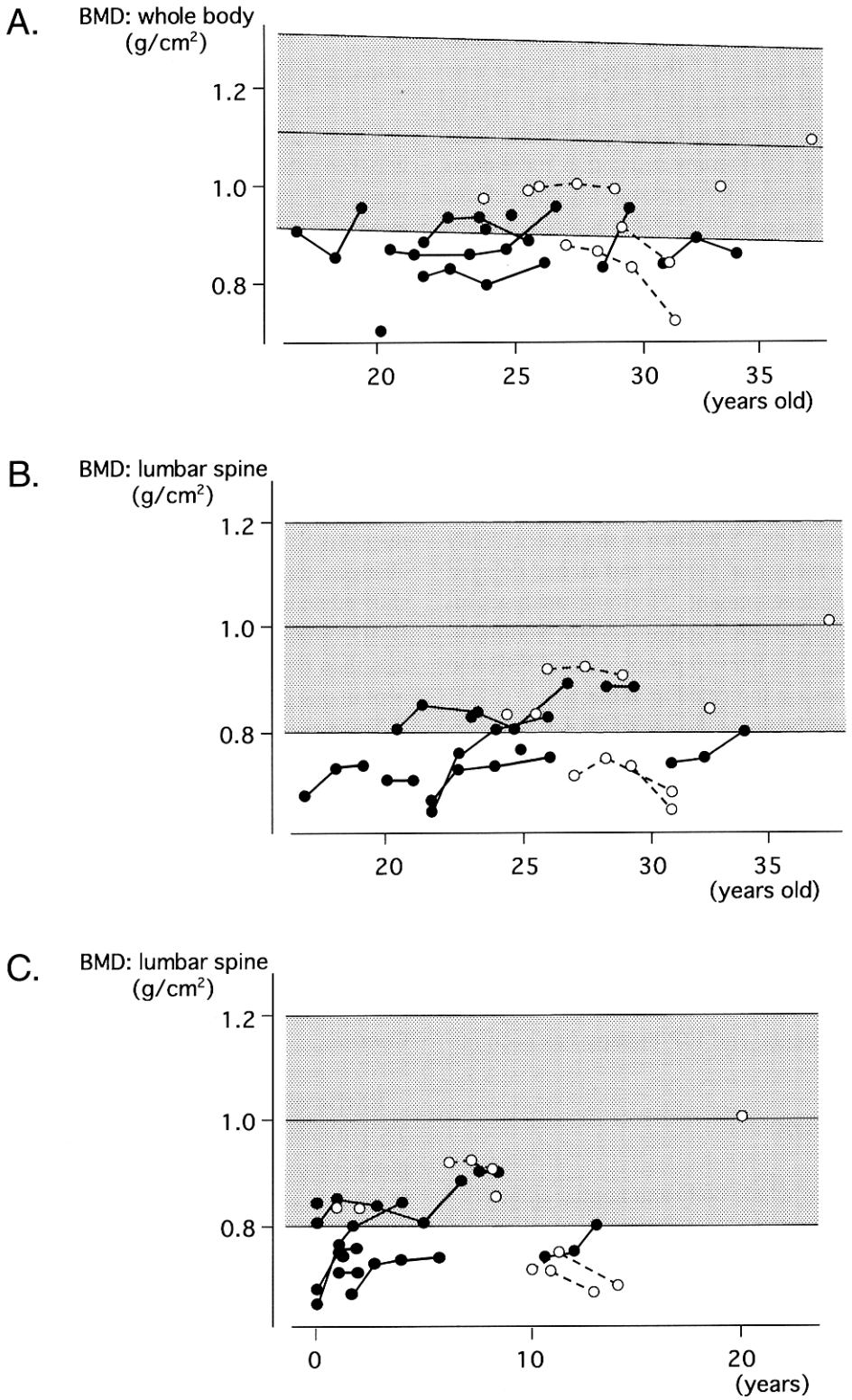

Fig. 1. Longitudinal changes of BMDs in TS subjects plotted by chronological age (A: whole body; and B: lumbar spine) and by length of E/P treatment (C: lumbar spine). Values in GH group (*solid circles*) and non-GH group (*open circles*) are represented separately. *Shaded areas* indicate normal range (mean ± 2 SD; whole body: 0.92–1.28 g/cm^2, and lumbar spine: 0.85–1.25 g/cm^2) determined from data of control women at each age in the study, and the *mid-line* shows the mean BMD level.

Table 2. Bone mineral density in TS patients

	GH group	Non-GH group
Whole body		
No. of BMD measurements	2.7 ± 1.5	1.9 ± 1.2
Mean age at BMD measurements (y.o.)	24.7 ± 4.3	29.3 ± 4.6
Mean duration of E/P therapy at BMD measurements (mos)	42 ± 46	107 ± 77
Mean BMD (g/cm^2)	0.88 ± 0.07	0.98 ± 0.08*
Lumbar spine		
No. of BMD measurements	2.8 ± 1.4	1.7 ± 1.0
Mean age at BMD measurements (y.o.)	24.8 ± 4.1	29.3 ± 4.6
Mean duration of E/P therapy at BMD measurements (mos)	42 ± 46	106 ± 77
Mean BMD (g/cm^2)	0.78 ± 0.06	0.84 ± 0.11

BMD: bone mineral density; y.o.: years old; mos: months, *P<0.05

The starting age for E/P therapy in the GH group also did not differ from that in the non-GH group.

However, the mean whole body BMD in the GH group was lower than the equivalent mean in the non-GH group, a result which is statistically significant (Table 2, $P = 0.031$). Although the differences in BMD of the lumbar spine between the GH and non-GH groups were not statistically significant ($P = 0.240$), the TS patients who received GH administration tended to have a lower lumbar spine BMD.

Discussion

Osteoporosis is a common complication in patients with TS [3–6], and may also be associated with severe bone fracture [6, 7]. In the present study, the BMD values of both the whole body and lumbar spine of TS patients were significantly lower than those in normal women. Some studies have also reported a reduction in BMD among TS subjects using DXA [7, 10].

Because a close relationship between estrogen deficiency and osteoporosis has been proven [11], it seems possible that the lifelong estrogen deficiency characteristic of TS might be the cause of osteopenia. Several studies on hormones and bone density in growing girls have documented the importance of estrogen [12–14]. Long-term E/P therapy initiated during mid to late adolescence and continuing indefinitely thereafter appears to be necessary to preserve normal peak bone mass.

Because most TS patients fail to initiate or progress through puberty, estrogen replacement for the purpose of secondary sexual development is usually begun at adolescence [15, 16]. Although estrogen is known to also be effective for bone mineralization in TS subjects [17–19], the optimal age to initiate estrogen therapy remains controversial because the potential sex steroids accelerate skeletal maturation and reduce final height [16]. In our present study, the E/P therapy was started at the age of 17–24 years after final height had been obtained with or without GH therapy. Although the E/P replacement was continued for a mean of 6.6 years, no clear increase in BMD was observed during the therapy. This result indicates that replacement of estrogen with progesterone in adult TS patients is not effective in increasing bone mineralization once peak bone mass was achieved. Through a longitudinal study, Shaw *et al.* [18] found that estrogen replacement in patients with TS is critical for preserving normal bone density. Mora *et al.* [19] reported that the TS subjects who started estrogen replacement before or by 11 years of age had better bone mineralization than those who started the treatment after age 12. One justification for early initiation of estrogen therapy has been the desire to minimize the decrease in bone mineralization. Also from our present study, estrogen with or without progesterone replacement to TS patients should be started at age of menarche in normal girls to gain sufficient bone mineralization. However, because estrogen has a contradictory effect on height growth, the adequate dose and/or period of estrogen administration must be determined by further prospective analyses.

Given that there was no apparent decrease in BMD during the course of E/P therapy, it can be argued that E/P therapy is effective in maintaining the bone mass of TS patients. In addition, from the present study the mean age at BMD measurement in the non-GH group was rather high compared to the GH group (Table 2, $P = 0.060$ in whole body and $P = 0.064$ in lumbar spine). The GH group also had a longer duration of E/P therapy during the BMD measurement period than the non-GH group (Table 2, $P = 0.079$ in whole body and $P = 0.083$ in lumbar spine). Although these differences were not statistically significant, the E/P therapy might have had a small effect on bone mineralization.

From these points of view, lifetime administration of cyclic E/P therapy for adult TS patients is important not only to induce menstruation and to render the uterus sufficient to support future conception with assisted reproductive technology, but also to prevent the decrease of BMD and the onset of osteoporosis.

Long-term GH therapy during childhood and adolescence may ameliorate the deficits in bone mass and improve the prognosis for more adequate peak bone mass. Saggese *et al.* [20] reported findings that suggest an important role for GH therapy in increasing the peak bone mass in children with classic GH deficiency. Also in patients with TS, Lanes *et al.* [21] assessed that BMD at the total body, femoral neck and lumbar spine in TS patients who were treated with GH was equivalent to that in a normal control group matched for height, bone age, weight and body mass index. Neely *et al.* [22] found that adolescents with TS who received GH were not osteopenic.

In contrast to the above results, our TS patients showed low BMD even when they received GH therapy. Especially, whole body BMD in the GH group was significantly lower than that in the non-GH group. These results indicate that GH administration during childhood and/or adolescence is not effective for bone mineralization. Bergmann *et al.* [23] also reported that treatment with GH increases osteoblastic function and height velocity in TS patients; however, bone mineral content in such patients never reaches normal levels, and an early effect of GH is to decrease spinal BMD. Although the reasons for the discrepancies are unclear, they may depend on differences in patient age, period of GH administration, and calculation of apparent BMD. Follow-up of TS patients to evaluate the risk of future osteoporosis is necessary.

Moreover, although TS patients whose GH administration started at ages of 10–19 years showed lower BMD than the patients of non-GH group in the present study, we cannot conclude that GH therapy has negative effects on bone mineralization. Through the early initiation of GH therapy, TS patients will be able to reach a sufficient final height at younger age, which implies that it will be possible to indicate an earlier start of E/P therapy and that a higher BMD will be gained by E/P administration. From these points of view, when short-stature girls are examined, diagnosis by karyotype analysis and treatment of TS patients need to be done aggressively as soon as possible.

Based on the present study, we can confidently state that the optimal protocol of hormonal replacement therapy with GH and E/P during childhood and adolescence should be established as soon as possible in order to maximize their growth in stature, uterine development and conception potential, and to minimize the reduction in BMD related to adult-onset osteoporosis.

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