Review article

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Bone health in children and adolescent with Turner syndrome

Abstract: Low bone mineral density (BMD) in patients with Turner syndrome (TS) has been reported in a considerable number of previous studies. Cortical and trabecular bone have been involved. Osteoporosis can be overdiagnosed in TS patients with a short stature unless BMD measurements are adjusted for body size. Optimization of bone health in girls with TS requires a healthy active lifestyle, including adequate calcium, vitamin D, and hormonal replacement therapy, according to consensus guidelines.

Keywords: adolescent; bone mineral density; children; Turner syndrome.

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Introduction

Bone mass or density has been measured by various techniques. These include conventional skeletal radiography, radiographic photodensitometry, single-energy absorptiometry, dual-energy X-ray absorptiometry (DXA), or quantitative computed tomography (QCT) (1). Bone mineral density (BMD) can be measured at appendicular or axial sites, such as heel and wrist or hip and spine, respectively (1). Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, with subsequent increase in bone fragility and ultimately fractures (1). Osteoporosis can be primary, as in osteogenesis imperfecta, or secondary, as in reduced mobility, systemic steroid, disordered puberty, malnutrition and low body weight, and inflammatory disorder (2). In young adult, osteopenia and osteoporosis were defined as BMD between 1 and 2.5 SDs and >2.5 SDs below the young adult mean, respectively (1). The presence of fractures and decreased bone mass are required to diagnose osteoporosis in children (2). In children, BMD measured by DXA scan can be affected by body size. Consequently, short individuals are likely to be misdiagnosed with osteoporosis, unless volumetric BMD (vBMD) is used (3). BMD measured by DXA represents areal density (grams per square centimeter) but not the volumetric density (grams per cubic centimeter) (4). vBMD, termed bone mineral apparent density (BMAD), of the lumbar spine has been calculated and used to account for differences in bone size (5).

This review of bone density in Turner syndrome (TS) will focus on the type of bone involved, relation to puberty and age, and risk of fracture. Management of abnormal BMD will also be discussed.

BMD in subjects with short stature

Those with short stature are likely to be misdiagnosed with osteoporosis. Volumetric adjustment BMD (vBMD), for body size, eliminates this issue (3). In 40 adult women with TS and 43 age-matched healthy women, those with TS had low areal BMD at the lumbar spine and femoral neck, compared with the control group. Women with osteoporosis, based on areal BMD, have a height <150 cm. vBMD at the femoral neck, but not at the lumbar spine, did differ significantly between patients with TS and the control group. The number of subjects with osteoporosis based on lumbar spine BMD fell from 8 to 2 when BMD was adjusted with respect to skeletal size (3). Areal BMD at the lumbar spine and femoral neck were positively correlated with height (3).

Cortical vs. trabecular bone

Cortical and trabecular comprise 80% and 20% of the skeletal mass, respectively. Cortical bone constitutes the outer part of all skeletal structures, whereas trabecular bone is

found in the end of long bones, throughout the vertebral bodies, and in the inner portions of the pelvis (6). Low BMD at the cortical bone had been reported in individuals with TS. Bakalov et al. (7) suggested that patients with TS experience a selective reduction in cortical BMD. In a study of 41 women with TS (aged 18–45 years) and 35 age-matched control women with premature ovarian failure but normal karyotype, those with TS experience a lower cortical BMD at the distal third of the radius compared with the control group. However, no significant difference in BMD at the ultradistal radius (trabecular site) has been noted between the two groups (7). It is suggested that the deficiency in BMD of the cortical bone in TS could be related to X-chromosome gene haplo-insufficiency rather than ovarian hormone exposure (7). A selective reduction in cortical BMD was confirmed by Holroyd et al. (8) in a study of 22 girls with TS (mean age, 12.7 years) and 21 girls without TS (mean age, 12.9 years), where those with TS had normal trabecular bone density with reduced cortical bone density compared with the control group (8). It was reported that the BMAD Z-score at the femoral neck and the cortical vBMD at the proximal radius, but not the BMAD Z-score at the lumbar spine, were low in subject with TS (8).

Data are conflicting, as a selective reduction in cortical BMD has been suggested (7, 8); however, low BMD can occur in the trabecular bone with or without cortical bone (9–13). In patients with TS, Khastgir et al. (9) reported that low BMD occurred at the lumbar spine and proximal femur in a study of 21 women with TS, aged 20–40 years. In a group of 54 patients with TS, aged 22–65 years (mean age, 37 years). Cleemann et al. (10) reported a decrease in T- and Z-scores at the spine and hip. However, BMD Z-score rather than T-score is widely used in pediatric population, Gravholt et al. (11) showed that vBMD scores decrease at the lumbar spine, but not at the femoral neck or forearm, in 60 women with TS (age, 37±9 years). The reduction in Z-score was more at the unloaded bones of the wrist, compared with the spine and femoral neck. Osteopenia and osteoporosis occurred in 28% and 23% of the subjects, respectively (11). Imbalance in bone remodeling was suggested, with increased bone resorption and unchanged or decreased bone formation markers (11). Högler et al. (12), in a group of 83 patients with TS (aged 4–24 years), showed that 15.8% and 28.4% of TS patients experienced low BMD Z-score at the lumbar spine and femoral neck, respectively. In 58 patients, aged 5–29 years, lumbar spine BMD Z-score lower than –1 SD and lower than –2.5 SD have been reported in 86% and 46.5%, respectively (13).

Low BMD at the cortical and/or trabecular bone occurred in patients with TS (7–13); however, normal BMD has been reported (14, 15). Normal vBMD values of the cortical and trabecular bone, measured by using phalangeal radiographic absorptiometry (PRA), have been reported in most of the 19 girls with TS (mean age, 13.3 years; range, 11.0–17.6 years) (14). In 68 girls with TS, age 2–11 years, bone age-adjusted vBMD SD scores at the cortical and trabecular bones, measured by using PRA, were within the normal reference range of healthy girls (15). However, in these patients, BMD was adjusted with respect to bone age, but not body size (15). In girls with TS, low bone mineral content (BMC) matched for age does not exist when it is matched for height age. Ross et al. (16), in a group of 78 girls with TS (4–13 years old) and 28 age-matched girls, showed that prepubertal-aged girls with TS may experience normal height age-adjusted BMC. The BMC of the wrist, adjusted for age and bone age, but not height age, and measured by single-photon absorptiometry, were decreased in the TS girls compared with the control group. Girls with TS experience low BMC at the spine, measured by dual photon absorptiometry (DPA), matched for chronological age, but not for bone age or height age (16).

Effect of pubertal development on BMD

Data with respect to the association between BMD and puberty are conflicting. Previous studies showed that there is a positive correlation between pubertal status and bone density (17). In 22 patients (age, 5.9–17.7 years) with TS, a positive correlation has been reported between pubertal status and trabecular BMD at the lumbar spine, but not cortical BMD at the midshaft of the femur (17). Compared with the controls, patients with TS experienced lower vertebral bone density and cortical bone area of the femur, after adjusting for weight, height, and skeletal age (17). However, these differences did not exist after additional adjusting for puberty. The decreased bone density of the vertebrae and cortical bone area of femur were attributed to estrogen deficiency (17).

vBMD values at trabecular bone sites, which are affected by estrogen, may be decreased in pubertal and postpubertal hypogonadal girls with TS and may deteriorate as the children get older with absence of pubertal development. In 19 patients with TS (mean age, 14.3 years), of whom 16 were receiving growth hormone (GH) therapy, and 45 control females (same mean age), the spinal BMD and BMAD in adolescent with Tanner breast stages 1 and 2 were greater in girls with TS, compared with the control group (18). However, this difference

does not exist in mid to late pubertal females (18). In 67 girls with TS (aged 6–19 years), Soucek et al. (19) reported that a decrease in trabecular vBMD occurred in the pubertal and postpubertal groups, but not in the prepubertal group; however, cortical vBMD decreased in all groups (19), where trabecular vBMD Z-scores were −0.2, −0.7, and −1.4 in the prepubertal, pubertal, and postpubertal groups, respectively, whereas the respective values of cortical vBMD were −2.0, −1.6, and −1.0, respectively. Soucek et al. (19) suggested that trabecular vBMD, which is affected by estrogen, may be decreased during and after puberty in hypogonadal TS girls (19). In 22 prepubertal girls with TS (mean age, 9.8±2.5 years), Aycan et al. (20) showed that the lumbar spine BMD and vBMD mean (SD) Z-scores were −1.2±1.2 and −0.8±−1.6, respectively, at the onset of GH treatment (20), where it was demonstrated that in prepubertal girls with TS, at the onset of GH treatment, lumbar spine vBMD mean Z-scores were −0.1±1 SD and −1.7±1.7 SD in children aged <11 years and ≥11 years, respectively, with pretreatment and 1 year posttreatment vBMD Z-score being higher in those <11 years old (20). Aycan et al. (20) found that prepubertal girls with TS may experience low vBMD Z-scores, which may deteriorate as the children get older and puberty does not commence (20).

Although it was reported in previous studies (17–20), no association between BMD and puberty was found by Högler et al. (12) in a group of 83 patients with TS (aged 4–24 years), where the mean vBMD Z-scores at the lumbar spine and femoral neck and BMC Z-score did not differ significantly among the prepubertal, pubertal, and postmenarchal groups in patients with TS (12). In this group of patients, total body BMC increased significantly in subjects developing puberty compared with subjects remaining prepubertal (12).

Whether spontaneous pubertal development is associated with a higher BMD is unclear, with an association found in some previous studies (13, 21, 22), but not in others (12). In individuals with TS, those with spontaneous puberty may experience higher BMD at the lumbar spine, compared with those requiring pubertal induction. In 37 patients with TS, 9 of whom had spontaneous puberty with menarche at the age of 12.55±1.17 years and 28 had induced puberty (21). In the latter group, estradiol therapy was received 2 years before menarche, which occurred at age of 14.68±0.63 and 14.47±0.53 years in 18 and 10 girls, respectively (21). In these patients, normal BMD and osteopenia at the lumbar spine were observed in those with spontaneous and induced puberty, respectively (21). Calcium intake, physical activity, karyotype distribution, and GH therapy did not differ between the groups (21). In 57 patients with TS, aged 16–54 years, patients with spontaneous menstrual cycle experience higher BMD at the lumbar spine, compared with those with primary amenorrhea (22). In 58 patients, aged 5–29 years, Costa et al. showed that patients with spontaneous puberty experience higher lumbar spine BMD, compared with those with absent spontaneous pubertal development (13). In patients with induced puberty, higher BMD Z-scores have been reported in those who received estrogen therapy for more than 2 years (13). In 83 patients with TS (aged 4–24 years), DXA results did not differ significantly between individuals with spontaneous and those with induced puberty (12).

Relation between BMD and age

A negative association between vertebral BMD and age has been reported in prepubertal girls with TS, but not in pubertal and postmenarchal group. This was observed by Högler et al. (12) in 83 patients with TS (aged 4–24 years), where lumbar vBMD Z-scores are negatively associated with age in prepubertal subjects, but not in the pubertal and postmenarchal group. In this group of patients, no association between age and vBMD at femoral neck has been observed (12). In 22 patients (mean age, 11.9±3.3 years) with TS, a negative correlation between age and vertebral BMD, but not femoral BMD, especially after the age of 10 years, was reported. However, a positive association was recorded between vertebral BMD and age in the control group (17). There was a significant difference in Tanner breast stage between the two groups, with stage 1 occurring in all patients with TS and with stage 2 or a more advanced stage occurring in 77% of the individuals in the control group (17). In a group of 58 patients with TS, aged 5–29 years, in those with non-pubertal stage, the lumbar spine BMD was negatively associated with age and height and positively associated with weight and bone mass index Z-scores (13). Those with spontaneous pubertal development have higher BMAD (13). In 22 prepubertal girls with TS (mean age, 9.8±2.5 years), lumbar spine vBMD Z-scores were lower in girls >11 years than those aged <11 years, with osteopenia becoming apparent in TS patients at pubertal age (20).

Risk of fracture

Patients with TS may experience a 25% increase in fracture risk (23). Medium- or high-impact trauma is reported in most of the fractures (23). Fractures were reported in

the cortical and trabecular bones. Patients with TS experience fractures at the appendicular skeleton (3), wrist (16), forearm (23, 24), arm (25), femur neck (25), and vertebra (25, 26). In a study of 78 girls with TS (4–13 years old) and 28 age-matched control, Ross et al. (16) showed that wrist fractures are higher in girls with TS compared with the control group, with an annual incidence rate of 9.1 of 1000 in the former and 3.5 of 1000 in the latter. In these patients, the relationship between wrist fracture and bone density remained unclear (16). However, the association between the risk of fracture and BMD is inconclusive in patients with TS (16, 19). In a study of 67 patients with TS (aged 6–19 years), the total vBMD at the metaphysis was lower in girls with a history of fractures compared with those without a history of fracture (19). Based on answers to questionnaires by 322 patients (age range, 1–73 years) and 1169 controls (age range, 2–82 years), patients with TS (especially those with a history of parental fractures and without spontaneous menstruation) experience an increased risk of fractures (24). These occurred more frequently at the forearm and at an early age (53±2 years) in those with TS, compared with the control group (24).

In patients with TS, osteoporosis and fractures are related to age and can be prevented by estrogen-replacement therapy. In a group of 70 women with TS (mean age, 31±12 years), Landin-Wilhelmsen et al. (25) showed that 13 fractures (7 arm or wrist, 4 femur neck, and 2 vertebral) occurred in 16% of the women with TS, of whom 50% were older than 45 years. In these women, both fractures and osteoporosis occurred in 5.7% of women with TS (>45 years old), and none of the women with osteoporosis received continuous estrogen substitution, although the age when the estrogen was started is unclear (25). The protective effect of estrogen on bone fractures was confirmed by Hanton et al. (26) and Bakalov et al. (3). In a group of 50 women (aged 30–59 years) with TS, Hanton et al. (26) showed that vertebral compression fractures occurred in 18.7% of patients who were not using estrogen replacement treatment according to consensus guidelines, compared with 0 patient in the adherent group. In a group of 40 adults with TS (age, 34±11 years) who had hormone replacement therapy (HRT) and 43 age-matched healthy women, Bakalov et al. (3) reported that the prevalence of osteoporosis and fracture did not differ significantly between the patients and the control group. In these patients, puberty has been induced by age 16 years in most and GH was given to 10 patients at age of 6–15 years (5). Fractures frequently occurred at the appendicular skeleton in patients with TS and the control group (3). Based on the fracture history of 177 women with TS (aged 19–60 years), fractures occurred in 32% of patients (27). In these patients, low BMD and hearing impairment, particularly the conductive type, are associated with increased risk of fractures (27).

Management

Physical exercise and a healthy diet with adequate calcium and vitamin D intake are essential to optimize bone health (28). Adequate calcium and vitamin D intake is recommended because many women have low levels of vitamin D (28). High body mass index should be avoided to minimize the risks of hypertension and insulin resistance. Vitamin D and/or calcium supplementations should be considered in patients with vitamin D deficiency and/or low calcium intake, respectively. Proper estrogen treatment improves BMD and is the mainstay of bone protection. Hormonal replacement therapy according to the international recommendation is the mainstay of bone protection in patients with TS (28). Patients with TS who experience osteoporosis and at risk for fractures should be commenced on the usual medical treatment for osteoporosis (28). In children with osteoporosis, a multidisciplinary approach is needed, with input from the pediatrician, physiotherapist, and occupational therapist (2). Physical activity can have a beneficial effect on bone density (29). Improvement in bone density occurs with HRT in men with hypogonadism (30). The effects of HRT on TS are discussed below. Bisphosphonate has been used in children with sustained osteoporotic fractures (31).

Physical fitness

Physical fitness is beneficial for BMD and cortical thickness. In a group of 50 women with TS, aged 21–45 years, a positive correlation between physical fitness and BMD at the forearm, BMC at the lumbar spine, and cortical thickness has been noted (32). Advice on appropriate exercise to maintain bone health is recommended, with respect to low maximal oxygen uptake in a group of 54 patients with TS (mean age, 37 years) (10). These patients experience decrease in T- and Z-scores at the spine and hip (10).

Calcium and vitamin D

Adequate calcium and vitamin D intake is essential for bone health. Calcium intake of >1000 mg and 1200–1500 mg of elemental calcium daily in the preteen years and after

11 years old, respectively, is recommended by the Turner Syndrome Study Group (28). Calcium and vitamin D3 supplementation in TS patients with low levels of 25-hydroxy-vitamin D [25(OH)D] is beneficial for lumbar spine bone density. As demonstrated by Cleemann et al. (10) in 54 patients with TS aged 22–65 years (10), HRT and calcium and vitamin D3 supplementation increases lumbar spine BMD and T-score; however, BMD at the distal third of the radius decreased, but remained unchanged at the forearm, ultradistal radius, and hip.

Patients with TS have normal osteoblast function, but not renal vitamin D metabolism. In 14 patients with TS (mean age, 12.6±5.9 years) and 15 age-matched controls, Saggese et al. (33) showed that osteoblast function, which was assessed by measuring the serum osteocalcin levels in response to 1,25-dihydroxy-vitamin D3 [1,25-(OH)2 D3] administration, did not differ between patients with TS and the control group (33). However, patients with TS experience an alternation in the physiological response of renal vitamin D metabolism, compared with the control group. In response to low calcium diet, an increment in serum 1,25-(OH)2 D levels has been observed in the control group but not in patients with TS (33).

Estrogen and vitamin D receptor genotypes

The association between *Pvu*II and *Xba*I ER-α polymorphisms and BMD in patients with TS has been examined by Sowińska-Przepiera et al. (34) in 32 patients with TS aged 17–38 years and 82 healthy controls. When analyzing *Pvu*II and *Xba*I polymorphic variants of the ER-α, patients with haplotypes other than XXPP experience an increase in BMD Z-scores after 2 and 4 years of estroprogestagen treatment (34). Peralta López et al. (35) examined the relationship between bone mass and vitamin D receptor genotypes in 65 patients with TS and 110 control subjects. With respect to *Apa*I sites, genotypes AA and Aa were predominantly reported in control and patients with TS groups, respectively. Lower BMD values were reported in patients carrying genotype bb (*Bsm*I) or ff (*Fok*I), compared with other genotypes. It was suggested that the *Bsm*I and *Fok*I polymorphic sites of the vitamin D receptor are genetic determinants of bone density in patients with TS (35).

To our knowledge, no previous study has examined the relationship between calcium supplementation and the cardiovascular and cerebrovascular events in patients with TS. Although previous studies in postmenopausal healthy women showed that calcium supplementation could be harmful to the heart, evidence is inconclusive. The association between calcium supplementation, with or without vitamin D, and the increased risk of cardiovascular events has been reported in postmenopausal healthy women (36, 37). However, this association has been rejected by a number of studies (38–41). Patients with TS are at an increased risk for heart anomalies. Hence, the relationship between calcium supplementation and cardiovascular or cerebrovascular events in patients with TS will need to be explored further with a prospective cohort of large numbers of patients.

Growth hormone

The positive effects of GH and estrogen supplementation on cortical as well as trabecular BMD have been reported by Sass et al. (14) and Sas et al. (15). In a study of 19 girls with TS (mean age 13.3 years) using phalangeal radiography, the mean vBMD SD scores of the cortical and trabecular bones increased significantly during GH treatment in combination with low-dose estrogens. This effect was maintained 3 years after the discontinuation of the GH treatment and with the use of estrogens in an adult dosage (14). Sas et al. reported that the bone age-adjusted vBMD SD score at the cortical and trabecular bones (measured by using PRA) increases significantly in 68 girls (aged 2–11 years) with TS during 7 years of GH treatment (15). No significant effect of low-dose estrogens in the last 3 years of the study period on the increment in BMD SD score has been reported (15).

Whether the effect of GH is predominantly on the cortical and/or trabecular bone is unclear. Data are conflicting, with a positive effect on cortical and trabecular (14, 15) BMD (but not BMC), total BMD and cortical thickness (42), total and lumbar BMD (but not total and lumbar BMC) (18), and cortical (but not trabecular) BMD (19). GH has a positive effect on trabecular vBMD, as measured by peripheral QCT (pQCT). This was observed by Bechtold et al. (42) in 21 TS patients (19.5±2.3 years old), of whom all had been received GH therapy for a mean period of 4.6 years and 20 were receiving estrogen supplementation. These patients experience low total vBMD, BMC, and cortical thickness at the radial metaphysis and diaphysis, compared with the controls. However, normal trabecular vBMD of the metaphysis has been noted in these patients (42). GH has a positive effect on lumbar spinal and total body BMD and BMAD, but not BMC, as measured by DPA. In 19 patients with TS (mean age, 14.2 years) and 45 age-matched controls, of whom 16 of 19 received GH therapy for a mean duration 3.2 years, Neely et al. (18) showed that girls with TS experience low mean lumbar spinal and total body BMC compared with the control group. However, no

	Population	Intervention	Measurement	Outcome	Comments
Neely et al., 1993 (18)	19 Patients with TS and 45 age-matched control; mean age, 14.3 years	16/19 Patients received GH therapy; BMD was reassessed after a mean interval of 1.3 years in 10 of the patients	Spinal and whole-body BMC, BMD, and BMAD	Patients who received estrogen experienced higher percentage increases in mean follow-up spinal BMD and BMAD, compared with untreated patients	Estrogen therapy benefits BMD at the lumbar spine in patients with TS
Mauras et al., 1997 (45)	7 Girls with TS; mean age, 12.5 years	Estrogen supplementation for 4 weeks	Effect of estrogen on calcium and bone turnover	Calcium absorption and retention increased and whole-body calcium turnover decreased	Estrogen therapy increases calcium absorption
Sass et al., 2000 (14)	19 Girls with TS; mean age, 13.3 years; range, 11.0–17.6 years	Assessment of the effect of GH treatment in combination with low-dose estrogen on BMD Estrogens was further increased to adult dosage after discontinuation of GH	Mean vBMD SD scores of the cortical and trabecular bone using PRA vBMD was repeated 3 years after discontinuation of GH treatment	vBMD at both sites increased during GH treatment in combination with low-dose estrogens and continued to be as high as in young healthy girls 3 years after discontinuation of GH treatment and the use of estrogens in an adult dosage	Estrogen increase cortical and trabecular vBMD
Khastgir et al., 2003 (9)	21 Women with TS; age range, 20–40 years	HRT's [S/C estrogen (E2) implants, with oral medroxy progesterone] effects on bone mass	Cancellous bone volume (%) and BMD at the lumbar spine and proximal femur	T-score at both sites improved from osteopenic levels before therapy to normal levels after 3 years of therapy	HRT has a positive effect on cancellous bone volume (%), bone wall thickness, and BMD at both sites
Hanton et al., 2003 (26)	50 Women with TS; age range, 30–59 years	Assess the effects of estrogen replacement treatment adherence vs. non-adherence	Assess BMD at the lumbar spine by DXA and QCT and vertebral compression fractures	Osteoporosis and vertebral compression fractures occurred in 37% and 18.7% of patients, respectively, in the non-adherent group No patients in the adherent group have osteoporosis	Taking estrogen replacement treatment as recommended protects from osteoporosis
Bechtold et al., 2001 (42)	21 TS patients; 19.5±2.3 years	Patients had received GH therapy, 20 patients were receiving estrogen supplementation	QCT to evaluate bone mass, density, geometry, and strength of the radial metaphysis and diaphysis	Normal trabecular vBMD of the metaphysis has been noted; however, BMC, total vBMD, and cortical thickness at the radial metaphysis and diaphysis decreased	Estrogen has a positive effect on trabecular BMD
Bakalov et al., 2003 (3)	40 Adult women with TS and 43 age-matched healthy women; age, 34±11 years	BMD was assessed in patients with TS who received hormone replacement therapy from midteens or the time of ovarian failure	Comparison of BMD and fracture history between patients with TS and control	Prevalence of fracture did not differ significantly between patients and control group Fractures frequently occurred at the appendicular skeleton in both groups	Prevalence of osteoporosis and bone fractures did not significantly increase in women with TS who are treated with estrogen therapy

(Table 1 continued)

	Population	Intervention	Measurement	Outcome	Comments
Landin-Wilhelmsen et al., 1999 (25)	70 Women with TS; mean age, 31±12 years; range, 16–71 years	Assessment of BMD and fracture in subjects with TS	Questionnaires, physical examination, BMD measurement by DXA	6/7 of women with TS who experienced osteoporosis were >45 years old; both fractures and osteoporosis occurred in 5.7% (>45 years old) of women with TS	Continuous estrogen therapy may prevent osteoporotic fractures None of the women with osteoporosis received continuous estrogen substitution
Kodama et al., 2012 (46)	54 Young TS patients	Assessment of adult doses of estrogen vs. low-dose estrogen vs. untreated Assessment of BMD during continuous estrogen therapy	BMD at the lumbar spine	BMD scores were higher in patients treated with adult-dose of estrogen, compared with the two other groups BMD increased during continuous adult-dose estrogen therapy	Estrogen has positive effect on trabecular BMD Negative correlation between BMD scores and the age of initiation of adult-dose estrogen therapy Those who treated before age 18 years experienced a higher annual gain in the BMD
Naeraa et al., 1991 (32)	50 Women with TS; age range, 21–45 years	Assess the relationship between estrogen therapy and BMC, BMD, and metacarpal dimensions	BMD and BMC	Direct correlation between duration of estrogen treatment and BMC at the lumbar spine, BMD at the forearm, and cortical thickness has been reported	Estrogen has a positive effect on bone health, with respect to duration of therapy
Benetti-Pinto et al., 2002 (47)	38 Women, 16 have TS and 22 have pure gonadal dysgenesis (GD) with 46,XX karyotype; age range, 16–35 years; mean age, 24.6 years	Estrogen therapy was started at mean age of 20.1±3.9 years	BMD at the lumbar spine BMD at femoral neck BMD at the lumbar spine and at femoral neck	Osteoporosis and osteopenia occurred in 91% with GD and 88% with TS Affected in 46% with pure GD and 69% with TS Positively associated with the length of estrogen therapy and BMI, respectively	Positive association between length of estrogen therapy and BMD at the lumbar spine

(Table 1 continued)

	Population	Intervention	Measurement	Outcome	Comments
Cleemann et al., 2009 (10)	54 Patients with TS; age range, 22–65 years, mean age, 37 years at baseline	Hormone replacement therapy and calcium and vitamin D3 [to those with low levels of 25(OH)D at baseline]	BMD T-score at spine and hip BMD at forearm, ultradistal radius, and hip BMD at the distal third of the radius and spine	Decreased at baseline and follow-up No significant changes at follow-up Decreased and increased, respectively, at follow-up	Estrogen has a positive effect on lumbar spine BMD
		Follow-up at 5.9±0.7 years of treatment	Bone formation markers Bone resorption markers	Did not change over time in TS Decreased over time in TS	
Sas et al., 2001 (15)	68 Girls with TS; age range, 2–11 years	Low-dose estrogens in the last 3 years of the study period, which was 7 years of GH treatment	BMD SD score at the cortical and trabecular bones by using PRA	No significant effect on the increment in BMD SD score	During a 7-year study of GH, low-dose estrogens in the last 3 years of the study period has no effect on the increment in BMD SD score
Costa et al., 2002 (13)	58 Patients; age range, 5–29 years	Evaluate the correlation of the Z-score of BMD with estrogen therapy in TS patients	BMD at the lumbar spine	Patients who received therapy for >2 years experience higher lumbar spine BMD Z-scores than who did not	Positive relation between estrogen duration and lumbar spine BMD
Soucek et al., 2011 (19)	67 Patients with TS; age range, 6–19 years	GH and estrogen therapy	vBMD at the radius using peripheral QCT Assessment of risk of fractures	Positive correlation between height-, age-, and cortical thickness-adjusted cortical vBMD and duration of GH and estrogen administration	No association between either GH or estrogen and trabecular vBMD at distal radius
Lanes et al., 1999 (48)	8 Girls with TS; mean age, 18.25 years	Continuous administration of conjugated estrogens and progesterone acetate for a 4.1±1.0 years	BMD of the femoral neck or lumbar spine	No change occurred over a period of 6.1 years after the intervention	Hormonal therapy has no effect on BMD

Table 1 Estrogen effects on bone density in patients with TS.

significant difference in mean lumbar spinal and total body BMD and BMAD has been reported between patients with TS and the control group (18). Patients who received estrogen experience higher percentage increases in spinal BMD and BMAD, but not whole-body BMD and BMAD, compared with untreated patients (18). No association between GH therapy and trabecular vBMD at the distal radius, as measured by pQCT, was reported by Soucek et al. (19). This study was performed in a group of 67 patients with TS (aged 6–19 years), who are being treated currently or in the past with GH and/or estrogens (19). In these patients, a positive correlation between the duration of GH therapy and estrogen administration and height-, age-, and cortical thickness-adjusted cortical vBMD, but not trabecular vBMD, has been reported (19).

No conclusive evidence on the effect of GH on BMD has been reported, whether a positive effect (14, 15, 18, 19, 42) or no effect (20, 43). Aycan et al. (20) showed that short-term GH supplementation has no effect on BMD at the lumbar spine in prepubertal girls with TS. In 22 prepubertal patients with TS (mean age, 9.8±2.5 years), no significant changes in BMD Z-scores and vBMD Z-score values at the lumbar spine before and 1 year after GH treatment has been reported (20). In a group of patients with TS (aged 12.3±2.9 years), 28 and 39 girls had never received GH and were treated with GH for at least 1 year (with an average GH treatment of 4.2 years), respectively. After adjustment for somatic size, BMD by DXA at the lumbar spine and distal third of the radius or phalangeal cortical thickness by hand radiography at the second metacarpal did not differ between the groups. GH effects were independent of estrogen exposure (43).

Estrogen therapy

The effects of estrogen on BMD are not related to karyotype (44). In 26 adult patients with TS and 12 adult women with pure gonadal dysgenesis, patients who received long-term estrogen replacement therapy, as recommended, experienced a decrease in markers for bone turnover, compared with untreated or insufficiently treated groups (44). The effects of estrogen on BMD in patients with TS are presented in Table 1.

Bisphosphonate therapy

No conclusive evidence regarding the safety and efficacy of bisphosphonate in children has been reported (49). Bisphosphonates are antiresorpive and used in adults with osteoporosis. Marini (49) suggested that children with prolonged bisphosphonate therapy may experience brittle, rather than better, bones. The treated bone becomes stiffer and less resistant to fracture. Bisphosphonates or other antiosteoporotic pharmaceuticals are not recommended by the Turner Syndrome Study Group for treating osteopenia in young women with TS (28). However, the usual medical treatment according to consensus guidelines is recommended for osteoporotic patients at risk for fractures (28). The effect of bisphosphonate on bone density has been examined in 38 children with normal karyotype and with osteogenesis imperfecta and cerebral palsy (50). It has been shown that intravenous pamidronate improves BMD and Z-scores at the lumbar spine and at the femoral metaphysis adjacent to the growth plate (50). Martinez-Soto et al. (51) showed that 1 year of bisphosphonate therapy, in a dosage of 4 mg/kg/year or 9 mg/kg/year, increases BMD and reduces fragility fractures in 15 patients (aged 8–16 years) with non-osteogenesis imperfecta-related bone fragility. However, long-term effects of small doses of bisphosphonate need to be explored by large trials. In children with sustained osteoporotic fractures, bisphosphonate therapy has been used with potential positive effect (31). The risks of bisphosphonate should be addressed and discussed with the patients and their parents. These may include osteonecrosis of the jaw (52), osteopetrosis (53), gastrointestinal upset with oral therapy, and symptomatic hypocalcemia if used in an individual with vitamin D deficiency or hypoparathyroidism (2).

Conclusion

Reduced BMD is not uncommon in those with TS, and it is a major concern for health professionals and patients. The cortical and trabecular bones can be affected. Fractures associated with osteoporosis can occur in these patients. Those with short stature are likely to be misdiagnosed with osteoporosis, unless vBMD for body size is used. Whether spontaneous pubertal development is associated with a higher BMD is inconclusive. In girls with TS, spontaneous puberty may be associated with a higher BMD at the lumbar spine, compared with those with induced or absent pubertal development. However, no significant difference in DXA results between individuals with spontaneous and those with induced puberty has been reported. An association between BMD deficiency of the cortical bone and X-chromosome gene haplo-insufficiency has been suggested in patients with TS. The *Bsm*I and *Fok*I polymorphic sites of the vitamin D receptor can be genetic

determinants of bone density in patients with TS. The association between calcium supplementation and cardiovascular and cerebrovascular events has been examined in postmenopausal women but not in patients with TS. This association needs to be examined in patients with TS. Measures to optimize bone health include exercise, adequate intake of vitamin D and calcium, and HRT according to consensus guidelines are essential for bone health in patients with TS. The relationship between calcium supplementation and the cardiovascular and cerebrovascular events in patients with TS needs to be further explored with a prospective cohort of large numbers of patients.

Conflict of interest statement

The authors declare no conflicts of interest.

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