Original article

Klinefelter’s syndrome and bone mineral density: Is osteoporosis a constant feature?

Syndrome de Klinefelter et densité minérale osseuse : l’ostéoporose est-elle une caractéristique constante ?

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Available online 3 December 2010

Abstract

Objectives. – Data on bone mineral density (BMD) in Klinefelter syndrome (KS) are scarce and contradictory. The aim of the present study was to investigate BMD in patients with KS and in healthy controls with special attention to gonadal status.

Material and methods. – We investigated 26 patients with KS (30 ± 9 yr) who had never been treated with testosterone. Thirty-nine age-matched healthy males served as controls. We assessed BMD by performing dual energy X-ray absorptiometry and measured serum hormone levels, including total testosterone (T), free testosterone, estradiol (E2), leptin. The estrogen to androgen ratio (E2/T) was used as an indirect measure for aromatase activity.

Results. – No difference was found in BMD at femoral neck (1.06 ± 0.16 vs 1.04 ± 0.14 g/cm2), or at lumbar spine (1.00 ± 0.09 vs 1.03 ± 0.11) between patients and controls. Two patients and one control were classified as osteoporotic (T-score ≤−2.5). Compared with controls, patients had lower levels of T and free testosterone, similar E2 levels, and increased E2/T (P < 0.05). In KS patients, leptin was significantly higher and correlated positively with E2/T (r = 0.484, P = 0.02). E2/T correlated with femoral neck BMD (r = 0.566, P = 0.02), T and free T correlated with lumbar spine BMD (r = 0.433, P = 0.05 and r = 0.534, P = 0.05).

Conclusion. – Osteoporosis is not a constant feature in young patients with KS, even without testosterone substitution. The aromatisation of T into E2, related to adiposity, may contribute to the achievement and maintenance of normal BMD in some KS patients.

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Keywords: Klinefelter syndrome; Bone mineral density; Osteoporosis; leptin; Aromatase activity

Résumé

Objectifs. – Les données concernant la densité minérale osseuse (DMO) dans le syndrome de Klinefelter sont rares et contradictoires. Le but de ce travail était d’étudier la DMO et le statut gonadique de patients atteints de Klinefelter et de témoins.

Matériel et méthodes. – Nous avons évalué par absorptiométrie biphotonique la DMO de 26 patients atteints de Klinefelter d’âge moyen 30 ± 9 ans, n’ayant jamais reçu de testostérone et les avons comparé à un groupe témoin de 39 hommes sains appariés pour l’âge. Le bilan hormonal incluait testostérone totale (T), testostérone libre, estradiol (E2) et leptine. L’aromatisation de T en E2 a été évaluée indirectement par le rapport E2/T.

Résultats. – La DMO du Klinefelter ne différait pas de celle des témoins au niveau du col fémoral (1.06 ± 0.16 vs 1.04 ± 0.14 g/cm2) ni du rachis lombaire (1.00 ± 0.09 vs 1.03 ± 0.11). Deux patients et un témoin étaient ostéoporotiques (T-score ≤−2.5). Comparés aux témoins, les patients avaient des taux de T et testostérone libre plus faibles, d’E2 comparables et un rapport E2/T plus élevé (P < 0.05). Chez les patients, la leptine était significativement plus élevée, corrélait avec E2/T (r = 0.484, P = 0.02). E2/T corrélait avec la DMO du col (r = 0.566, P = 0.02) ; T et testostérone libre corrélaient avec la DMO rachidienne (r = 0.433, P = 0.05 et r = 0.534, P = 0.05).

Conclusion. – L’ostéoporose n’est pas constante chez les jeunes patients Klinefelter, même en l’absence de substitution androgénique. L’aromatisation de la T en E2 dans le tissu adipeux pourrait contribuer à l’acquisition et au maintien du capital osseux chez certains patients.

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Mots clés : Syndrome de Klinefelter ; Densité minérale osseuse ; Ostéoporose ; Leptine ; Activité aromatase
1. Introduction

Klinefelter syndrome (KS) is the most common sex chromosome disorder, affecting one in every 600 males [1]. Affected males carry one or more additional X chromosome, which results in male hypogonadism and impaired spermatogenesis. Despite its high frequency, this syndrome is often overlooked. Characteristic clinical features of adults include small, firm testes, sparse body hair, gynecomastia, tall eunuchoid stature, and highly variable neurological and cognitive perturbations. However, due to its unobtrusive phenotype, diagnosis may be delayed for many KS patients, as the disease may only be detected at fertility clinics because spermatogenesis is affected by the meiotic problems caused by the disease in all patients [2].

It is known that sex steroid hormones play an important role in the acquisition and maintenance of bone mass in males as well as in females. Male hypogonadism has been recognized as one of the major causes of secondary osteoporosis. Androgen deficiency leads to bone loss and contributes to osteoporotic fractures in men. It has also become evident in recent years that estrogens play an important role in male skeletal health [3]. KS is usually associated with osteoporosis [4–6]. However, measurements of bone mineral density (BMD) by dual-energy x-ray absorptiometry (DXA), “the gold-standard” method for the non invasive diagnosis of osteoporosis, are scarce in KS patients [7–10]. Some studies have reported loss of bone mass in affected patients despite androgen replacement therapy [7–10]. Others have found a normal bone mass in affected patients without testosterone substitution [8]. In the study by De Rosa et al. [9], it was also suggested that bone loss is a minor characteristic of KS compared to idiopathic hypogonadotrophic hypogonadism, while epidemiological studies have reported an increased risk of osteoporotic fractures in men with KS [11]. Therefore, we aimed to investigate BMD in a group of patients with KS and in healthy age-matched controls by DXA, with special attention to gonadal status.

2. Patients

The study concerns 26 adult males diagnosed with KS (47,XXY) and referred to our department for endocrine assessment. The mean age of these patients was 30 ± 9 yr (16–52). They were all Caucasian. In more than 50% of cases, infertility was the only reason to perform caryotype analysis. For all subjects, a detailed medical history was obtained. Previous hormonal treatment was carefully recorded. No patients had received testosterone supplementation at the time of investigation, or calcium supplementation, vitamin D or bisphosphonates treatment. No patients had a history of fractures. They were examined by the same physicians following a standardized procedure within the same setting. All patients were postpubertal at the time of the study including the youngest. Present gynecomastia or history of mastectomy were recorded in half the patients. Body weight, height and waist circumference were measured. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. A group of 39 healthy, age-matched males (students and members of hospital staff) was used as controls. Smoking defined as a minimum of one cigarette daily was not an exclusion criteria. All controls were free of medication. Informed consent was obtained from patients and controls.

3. Methods

3.1. Bone mineral density

Areal BMD (grams per square centimeter) was determined for lumbar spine (L1–L4) and proximal femur by DXA using a Hologic QDR4500 scanner (Hologic, Waltham, MA, USA) with coefficient of variation of 0.9 and 1.9%, respectively. Quality control scans were performed daily, using a manufacturer-supplied anthropometric spine phantom. The T- and Z-scores were estimated using mean BMD and S.D. values supplied by the Hologic equipment manufacturer for white men.

3.2. Assays

Morning blood samples were collected and processed the day of DXA for determination of serum total testosterone (T) (Siemens Medical Solution Diagnostics, Puteaux, France, interassay CV, 4.7%), free testosterone (Active free testosterone RIA, Diagnostic System Laboratories, Webster, USA, interassay CV, 11%), estradiol (E2) (Siemens Medical Solution Diagnostics, Puteaux, France, interassay CV, 6.7%) and SHBG (ImmunoTech, Marseille, France, interassay CV, 8.3%). The free estrogen index (FEI) was calculated as the ratio between E2 levels and SHBG. The estrogen to androgen ratio (E2/T) was used as an indirect measure for aromatase activity. Serum concentrations of leptin were determined by immunoradiometric assay (Diagnostic System Laboratories, Inc., Webster, Texas, USA., interassay CV, 6.6%). To assess bone turnover, we measured intact osteocalcin (RIA, Cis-Bio International, Gif-Sur-Yvette, France, interassay CV, 5.2%), which reflects bone formation, and serum levels of the C-telopeptide of type 1 Cross laps (ELISA, Nordic Bioscience Diagnostic, Denmark, interassay CV, 8.1%), which reflects bone resorption. Serum 25-hydroxyvitamin-D was measured by Diasorin, 25-OHD, Stillwater, USA (interassay CV, 11%). Detection thresholds were 0.1 mg/l for T; 0.18 pg/ml for free testosterone; 7 pg/ml for E2; 0.2 nmol/l for SHBG; and 0.1 mg/ml for leptin.

3.3. Statistical analysis

We compared the KS patients with the control group. All values are given as means with standard deviation. Student’s t test was used to compare continuous variables between cases and controls. When the variance equality was not assumed (Levene Test), we performed nonparametric statistics tests, such as the Mann-Whitney test. Relationships between continuous variables and BMD were tested by Pearson and Spearman correlation analysis. Stepwise multivariate regression analysis was used to determine the independent predictors of BMD. All computations were done using the software SPSS program version 10. Statistical significance was declared if the two-sided P value was less than 0.05.
4. Results

Anthropometric data, hormonal profile and bone markers are given in Table 1. Weight and waist circumference were significantly higher in KS patients, and there was no difference in height between the two groups. T and free testosterone were significantly lower in KS patients than in the controls \((P<0.001)\). Half of the patients had a T level within the normal range \((2.5–10)\), whereas one control (obese and father of two children) had a T level lower than 2.5 \(\mu g/l\). There was no significant difference in E2 levels between the KS patients and the controls, while the E2/T ratio was increased in the Klinefelter group \((P<0.05)\). The SHBG level tended to be lower in KS patients \((P=0.07)\), and leptin levels were significantly higher in the KS patients than in control subjects \((P<0.001)\).

Bone resorption, as assessed by CTX-1, was significantly increased in KS patients while bone formation, as assessed by osteocalcin, was not different between the two groups. The concentrations of 25-hydroxy-vitamin D were significantly higher \((P=0.08)\). The two osteoporotic patients (35 and 39 years old) were characterized by markedly low levels of T \((0.9 \pm 0.19 \mu g/l)\) and free testosterone \((2.05 \pm 1.06 pg/ml)\), while the osteoporotic control (50 years) had a normal T level \((6.2 \mu g/l)\), a low level of E2 \((10 pg/ml)\), a markedly decreased FEI \((0.13)\) and a low E2/T ratio \((1.61)\).

In patients KS, weight correlated negatively with T \((r=-0.55, P=0.006)\) and SHBG \((r=-0.436, P=0.04)\), and positively with FEI \((r=0.433, P=0.05)\), E2/T \((r=0.63, P=0.002)\) and leptin \((r=0.807, P<0.001)\). The E2/T ratio correlated positively with leptin \((r=0.523, P=0.03)\). Femoral neck BMD correlated positively with weight \((r=0.322, P=0.05)\), E2/T ratio \((r=0.566, P=0.02)\), 25-OH D3 \((r=0.4, P=0.02)\) and negatively with age \((r=-0.387, P=0.06)\). Lumbar spine BMD correlated positively with T \((r=0.433, P=0.05)\) and free T

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Anthropometric parameters, hormonal profile and bone markers in Klinefelter patients and control subjects. Data are given as mean ± S.D. (range).</th>
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</thead>
<tbody>
<tr>
<td>Anthropometric data</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>30 ± 9 (16–52)</td>
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<tr>
<td>Weight (kg)</td>
<td>83.8 ± 15.7(^a) (56–114)</td>
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<tr>
<td>Height (cm)</td>
<td>181 ± 7 (165–199)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.4 ± 4.6 (16.9–37.7)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>91.6 ± 11.2(^b) (73–113)</td>
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<tr>
<td>Hormonal profile</td>
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<tr>
<td>Testosterone (T) (µg/l)</td>
<td>3.32 ± 1.85(^b) (0.8–7.1)</td>
</tr>
<tr>
<td>Free testosterone (pg/ml)</td>
<td>9.02 ± 6.41(^b) (1.3–34)</td>
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<tr>
<td>Estradiol (E(_2)) (pg/ml)</td>
<td>21.82 ± 15.24 (10–76)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>28.45 ± 11.9(^b) (9.9–56)</td>
</tr>
<tr>
<td>Free–estrogen index</td>
<td>0.86 ± 0.72 (0.23–2.92)</td>
</tr>
<tr>
<td>E(_2)/T ratio</td>
<td>7.08 ± 4.92(^c) (2.13–21.7)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>20.88 ± 12.27(^a) (2–42)</td>
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<tr>
<td>Bone markers</td>
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<tr>
<td>Osteocalcin (ng/ml)</td>
<td>26.8 ± 17 (11–75)</td>
</tr>
<tr>
<td>CTX (ng/ml)</td>
<td>6981 ± 4281(^a) (850–15370)</td>
</tr>
<tr>
<td>25-hydroxyvitamin-D (ng/ml)</td>
<td>36.25 ± 18.38(^a) (9–78)</td>
</tr>
</tbody>
</table>

BMD: body mass index; SHBG: sex hormone binding globulin; CTX: C-telopeptide.

\(^a\) \(P<0.05\).

\(^b\) \(P<0.001\).

\(^c\) \(P=0.07\).

Table 2 | Bone mineral density, T- and Z-scores in Klinefelter patients and control subjects. Data are given as mean ± S.D. |
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Lumbar spine</td>
<td></td>
</tr>
<tr>
<td>BMD (g/cm(^2))</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>T-score</td>
<td>−0.73 ± 0.96</td>
</tr>
<tr>
<td>Z-score</td>
<td>−0.71 ± 0.93</td>
</tr>
<tr>
<td>Femoral neck</td>
<td></td>
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<tr>
<td>BMD (g/cm(^2))</td>
<td>1.06 ± 0.16</td>
</tr>
<tr>
<td>T-score</td>
<td>0.08 ± 1.17</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.14 ± 0.97</td>
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(r = 0.534, P = 0.05). The multivariate analysis identified testosterone as independent predictor of lumbar spine BMD.

5. Discussion

In early adulthood, the most frequent sign leading to diagnosis of KS is infertility. More than 50% of the patients in our study had been diagnosed on the basis of azoospermia. The clinical appearance of the untreated patients differed from controls in terms of weight and waist circumference. These findings are confirmed by the report of Bojesen et al. [12], which indicates that KS patients have a higher percentage of truncal fat mass than control subjects. In addition, Aksglaede et al. [13] have recently reported a significant increased body fat mass with normal lean body mass in children and adolescents with KS.

Adults with KS are characterized by mild impairment of Leydig cell function, resulting in mild androgen deficiency [14,15]. In the majority of adult KS patients, serum testosterone concentrations are below normal, but some patients show levels within the normal range [2,16]. From our own experience and another study [15], men with idiopathic hypogonadotropic hypogonadism or Kallmann syndrome have markedly lower testosterone levels than KS patients. In contrast to the low testosterone levels of the KS patients in this study, we confirmed that untreated KS patients have a normal serum concentration of E2 [12,16] and a higher E2/Testosterone ratio than those of normal men, suggesting an increased aromatisation of androgens into estrogens. The high percentage of patients developing gynaecostasia supports this finding. Furthermore, compared with the general population, men with KS also have increased mortality from breast cancer, which might be explained in part by abnormal estrogen levels [17].

Eighty-five percent of circulating estrogens in the male come from peripheral aromatization of androgen precursors in different tissues, including fat. In our study, leptin levels were higher in KS patients than in the controls. It has been established that leptin levels are correlated with the degree of fat mass and are closely related to fat distribution. In particular, subcutaneous abdominal fat mass is a major determinant of leptin concentration [18]. Leptin levels correlated positively with the E2/T ratio in our KS patients, which suggests that excess adipose tissue may play a role in the aromatisation of androgens into estrogens in these patients.

The main result of the present study is that KS patients have a bone mass that is not different from that of normal controls, both at the femoral neck and lumbar spine. In agreement with Luissetto et al. [8], we confirm that not all KS patients are at a high risk of developing osteopenia or osteoporosis in young adulthood, even in the absence of testosterone substitution. In some previous studies of adults with KS, an impaired BMD was noted both in patients who had never received treatment and in those for whom treatment was delayed, after the age of 20 years [4,10]. Based on a Danish register [11], males suffering from KS have an increased risk of osteoporotic fractures.

Several factors may explain the discrepancy between our results and those of previous reports. First, the KS patients in our study were mainly diagnosed on the basis of infertility and may have had a morbidity profile that was different from that of other KS subjects with a more severe phenotype. Normal BMD could account for a mild phenotype. Secondly, our results were obtained by performing DXA measurements, while other studies used a different, less precise technique, such as single-photon absorptiometry [4] or an ultrasound imaging device [10]. Thirdly, some reports have measured BMD only at the forearm site [5]. Fourthly, others have focused on T-scores for tracking bone density changes [10]. However, because of the dependence of the T-score on both the mean and standard deviation of the reference population, it is now established that the use of disparate reference populations on different densitometry systems leads to T-score differences, even when the measured bone density is the same [19]. Lastly, ethnic differences could play a role and should be taken into account when considering a report showing low BMD at the lumbar spine and femoral neck in Korean men with KS [20]. In line with our findings, in a study by de Rosa et al., bone loss was a minor characteristic of KS, while it was a distinctive feature of idiopathic hypogonadotropic hypogonadal men [9]. Furthermore, in adolescents with KS, it was shown that bone mineral content is normal in untreated boys at the age of puberty [13], suggesting that if low BMD is present, it could occur at a distance of puberty. The two osteoporotic patients in our study were 35 and 39 years old.

Reaching peak bone mass is dependent on normal pubertal development and thereby on normal sex hormone secretion. KS boys enter puberty at the expected time. After an initial normal adolescent increase, serum testosterone concentrations remain within the low normal range [14]. In pubertal KS boys, serum E2 is also high, with a tendency for high E2/T ratios [14]. Estrogens play a key role both in the acquisition of peak bone mass in young men and in bone loss in elderly men [3]. The additional action of testosterone on stimulating periosteal apposition accounts for the larger size and thicker cortices of the adult male skeleton [3]. As previously reported in men [21], we have found positive associations between estrogens and BMD in controls. In KS patients, femoral neck BMD correlates positively with E2/T. The ability to aromatize androgens into estrogens plays a significant role in the regulation of bone metabolism in elderly men [22]. Aromatase is the key enzyme in estrogen biosynthesis. Human models of aromatase deficiency were the first to demonstrate the critical importance of aromatization of androgens into estrogen for male skeleton [23,24]. The skeletal phenotype of aromatase-deficient men is characterized by tall stature, unfused epiphyses, high bone turnover and severe osteopenia despite normal testosterone levels. Estrogen therapy in these subjects is associated with a marked increase in bone mass. In the aromatase excess syndrome [25], a clinical condition in which patients present excessive androgen to estrogen conversion, males develop prepubertal or peripubertal gynaecomastia and have greatly advanced bone age and short stature, indicative of the potent effect of estrogens on epiphyseal closure in the male sex as in the female sex. Moreover, an upper limit of bone density despite hypogonadism has been reported in affected male subjects [26]. Compared with controls, Klinefelter patients in our study had low levels of T and free testosterone, and maintained normal E2 levels. Recent clinical evidence suggests...
that a threshold exists for estrogen effects on bone in men [27]. In the MrOS Sweden cohort, Mellstrom et al. [28] have found that free serum estradiol was independent of free testosterone, a predictor of fractures. Moreover, men with low serum E2 levels had an increased risk of fractures, independent of testosterone status. By contrast, subjects with low T levels but normal E2 levels were not at higher risk of fracture. The inverse relationship between serum E2 levels and fracture risk was nonlinear, with a strong relation below 16 pg/ml.

Finally, compared with controls, our patients displayed a better vitamin D status which could have favourably affected their bone mass [29].

In conclusion, osteoporosis is not a constant feature in young patients with KS, even without testosterone substitution. Differences in BMD might form part of the spectrum of KS. Aromatisation of T into E2, which is related to excess adipose tissue, may contribute to a certain extent to the achievement and maintenance of normal bone mass in some patients with mild androgen deficiency.

Conflict of interest statement

The authors declare that they have no competing interests.

References