High prevalence of radiological vertebral fractures in adult patients with Ehlers–Danlos syndrome☆

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ARTICLE INFO

Article history:
Received 20 June 2015
Revised 12 November 2015
Accepted 17 December 2015
Available online 18 December 2015

Keywords:
Vertebral fractures
Ehlers–Danlos syndrome
Bone mineral density
Collagen
Osteoporosis

ABSTRACT

Previous studies have reported an increased prevalence of osteoporosis in Ehlers–Danlos syndrome (EDS), but these were limited by a small number of patients and lack of information on fragility fractures. In this cross-sectional study, we evaluated the prevalence of radiological vertebral fractures (by quantitative morphometry) and bone mineral density (BMD, at lumbar spine, total hip and femoral neck by dual-energy X-ray absorptiometry) in 52 consecutive patients with EDS (10 males, 42 females; median age 41 years, range: 21–71; 12 with EDS classic type, 37 with EDS hypermobility type, 1 with classic vascular-like EDS, and 2 without specific classification) and 197 control subjects (163 females and 34 males; median age 49 years, range: 26–83) attending an outpatient bone clinic. EDS patients were also evaluated for back pain by numeric pain rating scale (NRS–11). Vertebral fractures were significantly more prevalent in EDS as compared to the control subjects (38.5% vs. 5.1%; p < 0.001) without significant differences in BMD at either skeletal sites. In EDS patients, the prevalence of vertebral fractures was not significantly (p = 0.72) different between classic and hypermobility types. BMD was not significantly different between fractured and non-fractured EDS patients either at lumbar spine (p = 0.14), total hip (p = 0.08), or femoral neck (p = 0.21). Severe back pain (≥7 NRS) was more frequent in EDS patients with vertebral fractures as compared to those without fractures (60% vs. 28%; p = 0.04). In conclusion, this is the first study showing high prevalence of vertebral fractures in a relatively large population of EDS patients. Vertebral fractures were associated with more severe back pain suggesting a potential involvement of skeletal fragility in determining poor quality of life. The lack of correlation between vertebral fractures and BMD is consistent with the hypothesis that bone quality may be impaired in EDS.

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1. Introduction

Ehlers–Danlos syndromes (EDS) are a heterogeneous group of connective tissue disorders clinically characterized by skin hyperextensibility, articular hypermobility, and tissue fragility affecting skin, ligaments, joints, blood vessels, and internal organs [1]. Six types of EDS are reported in the Villefranche nosology: the classic type with prevalent cutaneous and articular involvement is due to mutations in COL5A1 or COL5A2 genes encoding type V collagen; the hypermobility type, also known as joint hypermobility syndrome, with prominent musculoskeletal features is without known molecular defects; the vascular type with vascular and internal organs fragility is caused by COL3A1 mutations leading to type III collagen defects; the kyphoscoliotic type with early onset progressive kyphoscoliosis is due to mutations in PLOD1 encoding lysyl hydroxylase 1; the arthrochalasis type with congenital bilateral hip dislocation is related to a specific set of mutations in COLIA1 or COLIA2 genes coding for type I collagen; and dermatosparaxis with pronounced skin fragility and redundance is caused by mutations in ADAMTS2 encoding procollagen I N-proteinase [2]. The most frequent EDS are the classic and hypermobility types, whereas the others are rare with variable clinical overlap [3]. Among these, the classic vascular-like type is an extremely rare EDS form characterized by cutaneous, articular and vascular involvement [4]. A subset of patients with overlapping phenotypes not fitting into the EDS types described so far are defined as not classified EDS [1].

Collagen types I, III and V, which are distributed in several connective tissues, i.e., skin, ligaments, tendons, blood vessels, and internal viscera are abnormal in EDS [5]. Type V collagen plays a central role in collagen fibrillogenesis and co-assembles with type I collagen to form heterotypic fibrils [6]. Abnormalities of type I and V collagen fibers may cause skeletal fragility by irregular arrangement of hydroxyapatite crystals and non mineralized collagen fibrils [7]. As a matter of

http://dx.doi.org/10.1016/j.bone.2015.12.007
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fact, it has been suggested that adequate quality collagen is required to form normally mineralized bone [8,9].

Preliminary studies reported that patients with EDS may have low bone mineral density (BMD) [10–15], whereas data on fragility fractures are limited to a few reports based on a retrospective historical assessment of the prevalence of clinical fractures [12,15]. Current knowledge supports the clinical relevance of radiologically diagnosed vertebral fractures [16], but they have been investigated in only a few EDS patients [11], being therefore still unclear the association of EDS with an increased risk of vertebral fractures.

In this cross-sectional study, we aimed at evaluating the prevalence of radiological vertebral fractures in a relatively large population of adult patients with different EDS types. Moreover, we aimed at studying whether radiological vertebral fractures were related to BMD and influenced the severity of back pain in patients with EDS.

2. Materials and methods

2.1. Subjects

Fifty-two consecutive patients with EDS (10 males, 42 females; median age 41 years, range: 21–71) were enrolled in the study. Inclusion criteria: 1) age older than 18 years; and 2) diagnosis of EDS. The clinical diagnosis of EDS classic and hypermobility type was based on Villefranche nosology [2]. Joint hypermobility syndrome, also known as EDS hypermobility type, was diagnosed according to thebright criteria [17].Sixteen patients were on treatment with drugs potentially affecting bone metabolism [18]. Specifically, 13 patients were treated with proton pump inhibitors, two patients with selective inhibitors of serotonin reuptake and one patient with anticonvulsivant drug (lamotrigine).

Exclusion criteria were: 1) treatment with anti-osteoporotic drugs, except for calcium and vitamin D; 2) prolonged immobilization; 3) spine trauma; and 4) previous surgical intervention on the spine except for correction of scoliosis in infancy [19].

The study was approved by the local ethical committee and the patients gave informed consent to the study and authorized the processing of their personal data according to Italian bioethics laws.

One hundred ninety-seven patients (163 females and 34 males); median age 49 years, range: 26–83) without family history and clinical evidence of EDS were enrolled as control group. The control subjects were retrospectively selected from a population of patients consecutively attending our bone outpatient units within the same period as that of EDS patients enrollment. The criteria used to select control subjects were: 1) comparable age to EDS patients; 2) comparable sex to EDS patients; and 3) availability of dual-energy X-ray absorptiometry (DXA) scans and spine X-rays. DXA scans were performed because of anamnestic/critical risk factors for osteoporosis. Spine X-rays were performed for the following reasons: DXA diagnosis of osteoporosis or BMD “below the expected range for age” or osteopenia with risk factors for fragility fractures, back pain and/or historical height loss. Exclusion criteria were: 1) previous or actual treatment with anti-osteoporotic drugs, except for calcium and vitamin D; 2) treatment with drugs known to cause osteoporosis [18]; and 3) history of chronic diseases causing secondary osteoporosis.

2.2. Methods

BMD of the lumbar spine, total hip and femoral neck was measured by DXA (Hologic Inc., Waltham, MA). Fractured vertebrae were excluded from the lumbar BMD analysis. DXA results were expressed in BMD (g/cm²). In aged 50 years or older subjects (21 patients with EDS and 79 control subjects), BMD was also expressed as T-score, comparing the results with those obtained in a sex-matched Caucasian population at peak of bone mass [20]. A T-score less than or equal to −2.5 SD at the hip or spine was defined as osteoporosis, whereas osteopenia was defined as a T-score between −1 and −2.5 SD. In younger than 50 years subjects (31 patients with EDS and 118 control subjects), the results were expressed as Z-score, comparing the results with those obtained in an age and sex-matched Caucasian population [20]. A Z-score less than or equal to −2.0 SD was used to define a BMD “below the expected range for age” [20].

Vertebral fractures were assessed by a quantitative morphometric approach [21]. Using a translucent digitizer and a cursor, six points were marked on each vertebral body to describe vertebral shape. Anterior (Ha), middle (Hm), and posterior (Hp) vertebral heights were measured and height ratios (Ha/Hp, Hm/Hp, Hp/Hp of the above vertebrae, and Hm/Hp of the below vertebrae) were calculated for each vertebra from T4 to L4; the fractures were defined mild, moderate and severe based on a height ratio decrease of 20–25%, 26–40% and more than 40%, respectively [21]. The morphometric analysis was performed by a single operator (M.D.). The intra-observer coefficient of variation, evaluated on a series of 10 measurements, was between 4% and 8%.

Pain was assessed using the numeric, 10-point, rating scale (NRS-11) in all patients; conventionally, severe pain was considered by a score over 7 [22].

For the molecular characterization of EDS patients, genomic DNA was extracted from peripheral blood leukocytes using standard procedures. Classic EDS patients carried either a COL5A1 or COL5A2 mutation; the classic vascular-like EDS patient carried the COL1A1 c.934C>T (p.Arg312Cys) mutation [4]. The EDS hypermobility type and the not classified EDS patients were not molecularly characterized. All of the exons and intron-flanking regions of the COL5A1, COLSA2 and COL1A1 genes were PCR amplified by using optimized genomic primer sets as previously described [4]. PCR products were bidirectionally sequenced using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA) and separated on an ABI 3130XL Genetic Analyzer (Applied Biosystems) [4].

Vitamin D status of EDS patients was retrospectively assessed using serum 25-hydroxyvitamin D values measured within 1 month before the enrollment and available in the clinical files. Hypovitaminosis D was defined by serum 25-hydroxyvitamin D values below 30 ng/ml.

2.3. Statistical analysis

All data were expressed as the median and range. Un-paired data were compared using Mann–Whitney test. Frequencies were compared using chi-square test with Fisher correction, when appropriate. A logistic regression model was used in the statistical analysis of risk factors for the occurrence of vertebral fractures. Statistical significance was assumed when p-values were equal or less than 0.05.

3. Results

EDS patients and control subjects showed no significant differences in BMD at lumbar spine (0.99 g/cm², range: 0.64–1.40 vs. 1.01 g/cm², range: 0.50–1.35; p = 0.70), total hip (0.85 g/cm², range: 0.54–1.30 vs. 0.88 g/cm², range: 0.72–1.30; p = 0.09) and femoral neck (0.79 g/cm², range: 0.55–1.10 vs. 0.90 g/cm², range: 0.51–1.00; p = 0.20). In 50 years or older EDS patients (21 cases), osteopenia and osteoporosis were found in seven (33.3%) and one (4.8%) patient, respectively, without statistically significant difference with respect to control subjects (43% osteopenia; 19% osteoporosis; p = 0.10 vs. EDS). In younger EDS patients (31 cases) and control subjects (118 cases), the prevalence of BMD “below the expected range for age” at either skeletal site was 32.3% and 24.6%, respectively (p = 0.49).

Patients with hypermobility EDS showed significantly lower femoral neck BMD (0.75 g/cm², range: 0.55–1.07) as compared to classic EDS (0.88 g/cm², range: 0.71–1.13; p = 0.01 vs. hypermobility EDS) and control subjects (0.90 g/cm², range: 0.51–1.00; p = 0.03 vs. hypermobility EDS), without significant differences in lumbar spine and total hip BMDs (data not shown).
Vertebral fractures were significantly more prevalent in EDS as compared to control subjects (Fig. 1), without significant difference between classic and hypermobility EDS (45.5% vs. 39.5%; \( p = 0.72 \)). Eleven patients had a single fracture, whereas in 9 patients two or more vertebral fractures were found. The fractures were mild in 14 patients, while the remaining 6 patients had moderate or severe fractures. In EDS patients, higher number per patient and more severe vertebral fractures as compared to control subjects were found (Fig. 1). Patients with vertebral fractures showed no significant differences in age, sex, type of EDS, BMD and serum 25-hydroxyvitamin D values as compared to patients who did not fracture (Table 1).

Stratifying the subjects for age, the prevalence of vertebral fractures was significantly higher in EDS patients as compared to control subjects either before (Fig. 2a) or after 50 years of age (Fig. 2b). Stratifying the subjects also for BMD, vertebral fractures were more prevalent in EDS patients with pathological BMD (i.e., osteopenia, osteoporosis or BMD below “the expected range for age”) with respect to patients with normal BMD (Fig. 2a,b). However, EDS patients with normal BMD maintained higher prevalence of vertebral fractures as compared to control subjects either before (Fig. 2a) or after 50 years of age (Fig. 2b). In detail, among patients younger than 50 years (31 cases), vertebral fractures occurred in 13 patients (41.9%) and only 6 of them showed BMD Z-score below −2 SD at either site. Among older patients with vertebral fractures (7 cases), three had normal BMD, whereas osteopenia and osteoporosis were found in three and one patient, respectively.

Thirty-nine EDS patients (75%) complained of back pain. In these patients, severe back pain (≥7 according to NRS-11 scale) was significantly associated with prevalence (odds ratio: 3.88, C.I.95% 1.17–12.48; \( p = 0.02 \)) and more severe (odds ratio: 2.51, C.I.95% 1.01–5.83; \( p = 0.03 \)) vertebral fractures. In fact, prevalence and severity of vertebral fractures were significantly higher in patients with severe back pain as compared to those with either moderate or absent back pain (Fig. 3).

Hypovitaminosis D was found in 42 patients (80.8%). At enrollment, 15 EDS patients with hypovitaminosis D were on treatment with vitamin D3, with (4 cases) or without (11 cases) calcium carbonate. Hypovitaminosis D was not correlated with vertebral fractures (odds ratio: 0.92, C.I.95% 0.22–3.78; \( p = 0.91 \)), BMD (odds ratio: 0.73, C.I.95% 0.16–3.35; \( p = 0.19 \)) and back pain (odds ratio: 1.1, C.I.95% 0.38–2.19; \( p = 0.83 \)).

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Table 1

Demographical and clinical features in Ehlers–Danlos syndrome (EDS) patients with vertebral fractures (VFs) as compared to those who did not fracture. Continuous data were presented as median and ranges and the comparisons were performed using non-parametric tests.

<table>
<thead>
<tr>
<th>Features</th>
<th>EDS patients without VFs</th>
<th>EDS patients with VFs</th>
<th>( p )-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>32</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40 (from 21 to 71)</td>
<td>42 (from 24 to 59)</td>
<td>0.55</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>7/25</td>
<td>3/17</td>
<td>0.54</td>
</tr>
<tr>
<td>EDS type (Classic/hypermobility)</td>
<td>6/23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar BMD (g/cm²)</td>
<td>1.05 (from 0.64 to 1.40)</td>
<td>0.87 (from 0.78 to 1.23)</td>
<td>0.14</td>
</tr>
<tr>
<td>Lumbar BMD T-score (SD)</td>
<td>−0.6 (from −3.5 to +2.1)</td>
<td>−1.0 (from −3.4 to +1.2)</td>
<td>0.17</td>
</tr>
<tr>
<td>Total hip BMD (g/cm²)</td>
<td>0.98 (from 0.69 to 1.30)</td>
<td>0.83 (from 0.54 to 1.03)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total hip BMD T-score (SD)</td>
<td>−0.2 (from −2.5 to +1.7)</td>
<td>−1.1 (from −3.2 to +0.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td>0.82 (from 0.59 to 1.10)</td>
<td>0.75 (from 0.35 to 0.96)</td>
<td>0.21</td>
</tr>
<tr>
<td>Femoral neck BMD T-score (SD)</td>
<td>−0.8 (from −3.5 to +1.6)</td>
<td>−1.1 (from −2.7 to +0.2)</td>
<td>0.13</td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D (ng/ml)</td>
<td>23 (from 5 to 70)</td>
<td>20 (from 7 to 87)</td>
<td>0.29</td>
</tr>
</tbody>
</table>
4. Discussion

This cross-sectional study reported for the first time high prevalence of radiological vertebral fractures in adults with EDS, even in the presence of normal BMD as assessed by DXA. The presence and severity of vertebral fractures were significantly associated with back pain in this clinical setting.

EDS refers to a group of heritable connective tissue disorders caused by alterations in different collagen genes and in genes encoding enzymes involved in maturation of fibrillar collagens leading to abnormalities in extracellular matrix in several systems and organs [1,2]. Approximately 90% of bone matrix is composed of collagen which serves as a tissue scaffold but also provides a substrate for cell anchorage and regulates bioavailability of growth factors and cytokines [23]. Adequate quality collagen is required to form normally mineralized bone. Moreover, collagen may regulate the function of bone cells and in experimental animals the lack of collagen was associated with an impairment of osteoblastogenesis [24]. The mechanism leading to skeletal fragility in EDS may be similar to those occurring in osteogenesis imperfecta, a disease due to a primitive defect in type I collagen synthesis [7]. In EDS, type I collagen is not frequently mutated but the defects of other types of collagen (e.g., type V collagen) may also lead to misalignment of type I collagen molecules and abnormal fibrillogenesis [6], with abnormal structure of extracellular bone matrix and possible secondary alterations in bone remodeling [25]. Another mechanism potentially involved in the skeletal fragility of EDS is hypomobility due to joint and muscle involvement. In fact, bone loss at proximal femur was shown to be closely correlated with levels of physical activity in EDS [13].

Over the last 20 years several studies have reported a variable degree of bone loss in patients with EDS [10–15]. Differently from previous studies, we provided an age-related densitometric definition of low BMD, since the majority of patients with the disease are relatively young. Interestingly, we observed that more than 50% of our patients had normal BMD. Indeed, osteoporosis was found in only one patient older than 50 years, whereas BMD “below the expected range for age” was observed in slightly more than one third of younger patients with EDS. These percentages were lower than those previously reported in smaller series [10,11] and in studies using different densitometric criteria [15]. Notwithstanding the relatively reassuring densitometric data, we observed high prevalence of vertebral fractures in patients with EDS. Vertebral fractures are the hallmark of osteoporosis and the radiological approach has emerged as the method of choice for evaluating their true prevalence in population studies [21]. Using this approach, our study reported for the first time vertebral fractures in more than 30% of patients with EDS, a similar percentage to that already reported in other conditions at very high risk of fragility fractures [26–30]. It is noteworthy that fracture risk in this study was not associated with patient age, with prevalence of vertebral fractures being high in both young and older patients. This finding is in agreement with previous studies reporting high prevalence of vertebral fractures in young adults with skeletal fragility [28–30] and consistent with the hypothesis that deterioration in bone quality and strength during the first decades of life may lead to early development of fractures in patients with secondary osteoporosis. Interestingly, prevalence of vertebral fractures was not different between classic and hypermobility EDS, suggesting that degree of bone damage may be similar notwithstanding the different genetic background, but in agreement with the similar and partly overlapping clinical presentation of these disorders. It is noticeable that in these two EDS types collagen and elastin alterations in skin biopsies are identical; in particular, irregular and fragmented collagen fibrils, and cauliflower fibrils as a hallmark of disturbed fibrillogenesis of the heterotypic type I/V collagen fibrils are described [31].

In our study, vertebral fractures were found even in patients with normal or low-normal BMD, such as already observed in other forms of secondary osteoporosis [32,33]. Indeed, BMD reflects bone quantity but not bone quality which is determined by structural and material properties [9]. Our study suggests that in EDS abnormal collagen synthesis may lead prevalently to impairment of bone quality. As a matter of fact, this hypothesis is consistent with previous observations that ultrasonometric bone parameters were shown to be compromised more than BMD in EDS patients [12].

We reported for the first time high prevalence of hypovitaminosis D in EDS patients. Indeed, malabsorption caused by collagen abnormalities may be a potential cause of hypovitaminosis D in patients with EDS [34]. In the general population, vertebral fractures were associated with hypovitaminosis D [35], especially when accompanied by secondary hyperparathyroidism [36]. Such an association was not observed in this study, possibly due to limited number of EDS patients with normal vitamin D values, lack of information on serum parathyroid hormone values associated with hypovitaminosis D, cross-sectional design of the study which did not allow to investigate the temporal relationship between untreated hypovitaminosis D and development of vertebral fractures. Moreover, the retrospective evaluation of vitamin D status did not allow to exclude possible inter-assay variability in 25-hydroxyvitamin D measurement.

Some limitations of our study merit mention. The cross-sectional design of this analysis did not allow to investigate the timing of development of vertebral fractures in EDS. Moreover, our control population demonstrated to be at relatively low risk of osteoporosis and fractures [37]. Therefore, large differences observed between our patients and controls may not reflect an incremental risk of fractures in EDS with respect to other high risk conditions [26–30,32,37], although the prevalence of vertebral fractures in our EDS patients was higher than that already reported in the general population [38,39]. Moreover, we did not measure biochemical markers of bone turnover [40] and we did not evaluate bone microstructure in relationship with prevalent vertebral fractures [12,21].

Besides the aforementioned limitations, the results of our study may be clinically relevant, since they suggest that skeletal fragility may be a frequent complication of EDS. The occurrence of vertebral fractures even in the presence of normal BMD suggests that DXA measurement may not well reflect the bone health status and that vertebral fracture assessment should be included in the diagnostic work-up of EDS, especially in patients with back pain which was shown to negatively impact on quality of life [41–43].

**Funding**

This study was partially supported by CROMO (Center for Research in Osteoporosis and Bone Metabolism), GIOSEG (Glucocorticoid
Induced Osteoporosis Skeletal Endocrinology Group, University of Brescia Italy and MIUR (Italian Ministry for University and Research).

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