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CLINICAL MANAGEMENT OF ENDOCRINE DISEASES

Skeletal fragility: an emerging complication of Ehlers–Danlos syndrome

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Abstract
Ehlers–Danlos syndrome (EDS) is an emerging cause of skeletal fragility. Mechanism of bone damage are probably multifactorial in line with the different skeletal phenotypes that can be found in clinical practice. A structured approach to clinical management of bone metabolic complication in EDS is proposed.

Keywords Osteoporosis · Vertebral fractures · Ehlers–Danlos syndrome · Bone mineral density · Bone structure

Introduction

Ehlers–Danlos syndrome (EDS) is a group of heritable connective tissue disorders mainly characterized by skin hyperextensibility, joint hypermobility, and tissue fragility [1]. The recently revised EDS classification includes 13 subtypes [2]. Among these, the classical (cEDS, prevalence 1:20,000) and the hypermobile (hEDS, prevalence 1:5000–15,000) are the most frequent forms. The other EDSs are very rare and clinically overlapping [3]. For several EDS forms, specific collagen mutations have been identified leading to different clinical expression of the various EDS. Molecular heterogeneity of the disease accounts at least in part for the differences among patients' clinical expression, leading, in turn, to misdiagnosis or frequently to late diagnosis in advanced age. The diagnosis of EDS should be supported by laboratory findings in all cases except hEDS, which is still a clinical diagnosis without any confirmatory test [2, 3].

The vast majority of cEDS patients, chiefly characterized by abnormal skin involvement and generalized joint hypermobility (gJHM,) bear heterozygous mutations in the genes encoding for the two chains of type V collagen (*COL5A1* and *COL5A2*) [4, 5]. hEDS follows an autosomal dominant inheritance pattern with an unknown molecular basis and is mainly characterized by joint instability complications, and minor skin changes [2, 3]. Recently, stringent clinical criteria for the diagnosis of hEDS have been introduced and those patients with symptomatic JHM not fulfilling these criteria have been recognized with the label of "hypermobility spectrum disorders" (HSD) [6] The clinically relevant component of this spectrum is now defined hEDS/HSD [7]. Early diagnosis of hEDS/HSD, based on articular signs such as capsule-ligamentous laxity, may prevent chronic complications of the disease (luxations and soft-tissue lesions, such as tenosynovitis and fasciitis) leading to chronic musculoskeletal pain [2, 3, 8]. Management of EDS is based on a multidisciplinary approach, in which dermatologists and orthopedic surgeons are joined by other specialists tackling the extra-articular manifestations of the disease, in which gastrointestinal, cardiovascular, neuro-psychiatric, ophthalmologic, and gynecological involvement may occur [2, 3, 8].

Interestingly, bone is not traditionally considered a target organ for EDS, despite reduced bone mass (BMD) being quite consistently reported in EDS since the 90s although in small studies [9–11]. Moreover, during the last years an increased risk in radiological vertebral fractures has been repeatedly found in reasonably large EDS patient cohorts [12, 13]. This finding is particularly important, since it has emerged as the landmark of bone damage not only in postmenopausal osteoporosis but particularly in secondary forms of osteoporosis [14–16].

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As a consequence of this cultural gap, neither a consensus on how to manage bone metabolic complications, or even on their real existence, has been reached so far nor diagnostic or therapeutic guidelines for this emerging complication of EDS have yet been published.

Therefore, based on the pathophysiology of the syndrome, on literature findings and our clinical experience, we attempted to provide a structured approach for the management of bone complications in EDS. To this end, we also reported bone data of two paradigmatic EDS patients with morphometric vertebral fractures.

Patients and methods

Patients

We report the bone data collected, with the methods reported below, during the routine evaluation of two EDS patients, after obtaining their informed consent, who are part of a cohort of 118 EDS/HSD patients (followed-up from January 2013 to December 2017) at the Endocrine Outpatient Clinic of the University Hospital of Brescia, Italy (Table 1).

Methods

Clinical and molecular diagnosis

Up to February 2017, EDS diagnosis was based on the Villefranche criteria [17] and nowadays on their revision according to the 2017 International Classification of EDS [2]. For the two cEDS patients here reported, confirmatory molecular diagnosis was performed as previously described [4, 5].

Bone evaluation

BMD at 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 g/cm^2. In aged 50 years or older 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 [16]. 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, the results were expressed as *Z*-score, comparing the results with those obtained in an age and sex-matched Caucasian population [18]. A *Z*-score less than or equal to −2.0 SD was used to define a BMD "below the expected range for age" [18].

Vertebral fractures were assessed on a thoracic and lumbar spine radiogram performed in the same time period of the DXA examination, by a quantitative morphometric approach as previously described [19]. Briefly, 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 Hp/Hp of the below vertebrae) were calculated for each vertebra from T4 to L4; the fractures were defined according to Genant et al. [19], mild, moderate and severe based on a height ratio decrease of 20–25%, 26–40%, and more than 40%, respectively.

Concomitantly with the DXA and X-ray examination the patients underwent blood and urinary tests for serum calcium and phosphorus, 24 h urinary calcium, PTH, 25(OH) vitamin D, and also bone turn-over markers: serum osteocalcin and alkaline bone phosphatase as markers of bone formation and serum CTx as marker of bone resorption.

Table 1 Clinical features of two EDS patients with vertebral morphometric fractures

Pt #	Sex	Age	Type	Markers	BMD	VFs	Vitamin D
1	F	18	cEDS	↑CTx	N	L1 mild[a]	N
2	F	34	cEDS	N	OPe	T8, L3 mild[b]	Low

N Normal, *mod* moderate, *Opo* osteoporosis, *Ope* osteopenia, *CTx* telopeptide

[a]During follow-up

[b]T8 at baseline, L3 during follow-up

Patient bone data

Case 1

Baseline

An 18-year-old cEDS woman (eumenorrhoic, BMI 30.4), previously published as AN_002507 and carrying a pathogenetic *COL5A1* variant [4], had normal BMD and no vertebral fractures at morphometric evaluation at presentation. Dietary intake of calcium was around 1000 mg/day. Serum calcium (9.8 mg/dl), phosphate (2.8 mg/dl) and PTH (13 pg/ml) were normal, with normal 25(OH) vitamin D values (34 ng/ml) without supplementation. Urinary calcium levels were under normal range for patient weight (76 mg/24 h). Only serum CTx was slightly increased (0.63 ng/ml vs normal range 0.1–0.45).

Follow-up

Two years later BMD persisted normal, but a mild lumbar spine deformity (L1) was discovered. Cholecalciferol

25,000 UI monthly and Calcium carbonate at a dose of 1000 mg daily, divided into two doses, were commenced (Table 1).

Case 2

Baseline

A 34-year-old cEDS woman (eumenorrheic, BMI 20.4), previously published as AN_002502 and carrying a pathogenetic *COL5A1* variant [4], presented with severe hypovitaminosis D (6.9 ng/ml) treated with Cholecalciferol 10,000 UI 4 drops/daily and with very low BMD (lumbar *Z*-score −3.4 DS; neck femoral *Z*-score −3.0 DS). She also showed a mild spine deformity (T8). Dietary intake of calcium was around 1000 mg/day. Bone markers were normal. Serum calcium (8.8 mg/dl), phosphate (2.8 mg/dl) and PTH (50 pg/ml) were normal. Also urinary calcium levels were normal for patient weight (149 mg/24 h). Cholecalciferol dose was increased (25,000 UI every 21 days) and Calcium carbonate 500 mg bid was initiated.

Follow-up

Four years later, despite persistently normal bone markers a normalized 25 (OH) vitamin D (32.5 ng/ml) and improved bone densitometric parameters (lumbar *Z*-score −2.2 DS; neck femoral *Z*-score −2.2 DS), a new mild spine deformity (L3) was discovered. Bisphosphonate treatment with Neridronate i.m was initiated (Table 1).

Rationale for bone evaluation in EDS

Pathophysiological basis

Structural aspects

The formation and maintenance of the skeleton requires progenitor cell recruitment, replication and differentiation, which are processes regulated in part by the extracellular matrix (ECM) [20, 21]. Approximately 90% of the matrix in bone is composed of collagen and, in particular, type I collagen is the main protein component of bone tissue in which it serves as a tissue scaffold, provides a substrate for cell anchorage, and regulates bioavailability of growth factors and cytokines. In addition, the ECM also directly regulates cell behaviors such as migration, differentiation, proliferation, and survival, through its ability to engage adhesion receptors as well as to regulate growth factor bioavailability and signaling [22].

The prototype of genetic disorders due to collagen defect, in which structural properties of bone are seriously impaired, is Osteogenesis Imperfecta (OI) [23]. OI is characterized by early and very frequent clinical and morphometric vertebral fractures [24]. In this disorder type I collagen mutations are strictly linked to bone fragility [25].

Different collagens, which are distributed in several connective tissues, i.e., skin, ligaments, tendons, blood vessels, and bone, are involved in the molecular pathology of almost all genetically defined EDS types [2, 3]. The key collagens directly involved in these diseases are type I, III, V, and XII, due to mutations in *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2*, and *COL12A1*. Furthermore, other EDS genes (i.e., *PLOD1, ADAMTS-2*, and *FKBP14)* encode proteins involved in collagen processing, folding and cross-linking. In addition, EDS genes including *B4GALT7*, *B3GALT6*, *CHST14*, and *DSE* are implicated in the biosynthesis of glycosaminoglycans that are known to impact collagen fibril formation and deposition into the ECM, as well as tenascin X encoded by *TNXB* [2]. Concerning hEDS/HSD, although the underlying molecular defect is still unknown, it was demonstrated that in vitro patients' fibroblast show a generalized extracellular matrix disorganization including collagen type I, III, and V, which could partly be explained by the high levels of the active form of the MMP-9 collagenase recently reported in culture media [26, 27]. Of note, type V collagen, which is found in skin, cornea, and bone, has a fundamental role in type I collagen fibrillogenesis, forming a core inside major collagen fibrils. Its deficiency in cEDS should result in the misalignment of the collagen molecules and fibers, which are required for adequate bone mineralization [28]. Therefore, type V collagen alterations may also be hypothesized to result in a reduction of bone resistance and osteoporosis [28]. So far, however, a precise genotype/bone phenotype correlation in EDS patients was not reported.

Clinical aspects

Multiple risk factors for skeletal fragility observed in EDS can be found including a possible contribution of reduced mobility and proprioceptive defect. In the following sections available literature data on the different bone endpoints in EDS are reported.

Bone markers Currently, very few data have been published on bone markers in EDS and their clinical relevance. Available literature suggests that independently of the markers considered (bone resorption vs bone formation markers) no difference seems to exist in EDS patients as compared with control group [13, 29]. However, in the study by Eller-Vainicher and co-authors [13] a trend statistically not significant towards an increase of serum telopeptide and a decrease of osteocalcin as compared with controls was found. It is possible that the small size of the

available studies did not allow to disclose an uncoupling between bone formation and resorption. Alternatively it is possible that in some ESD patients but not in all of them an uncoupling between bone resorption and apposition may be present, possibly due to a different genetic variants.

BMD Reports of reduced bone mass in the more common EDS variants were firstly published almost 25 years ago, in 1994, by two works with small series of cases. One analyzed lumbar and femoral BMD in 7 hEDS patients (2 M; 5 F) and found an important reduction at both sites [9]. The other work reported in 4 patients (3 M; 1 F) with hEDS a lumbar BMD reduction predominantly in trabecular bone, whereas BMD at femoral neck was similar to that of a control group [10]. In 2000 Carbone et al. reported significantly reduced femoral neck BMD as compared to controls. However, once age, weight and activity level were corrected for, the difference became not significant [29]. These discordant results may possibly be due to the small sample size of the above series and to the wide heterogeneity regarding age and gonadal status (both females in pre- and post-menopausal status were evaluated).

Fractures In 1998 Dolan et al. [11] described a surprising and important increase in prevalence (10 times) of common clinical fractures in 23 EDS patients with respect to the general population (86.9% vs 8.7%) in association with a reduced BMD. In 2017 Holick et al. [30] reported bone fragility fractures in infants with documented EDS.

Radiological morphometric vertebral fractures were reported to be significantly more prevalent in 52 EDS patients as compared to 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 cEDS and hEDS. BMD was not significantly different between fractured and non-fractured EDS patients at either site [12]. A subsequent Italian study confirmed these results in terms of prevalence of vertebral fractures; furthermore, the authors also reported an altered bone quality, as evaluated by TBS analysis in fractured vs non-fractured EDS patients (1.245 ± 0.138 vs 1.325 ± 0.086, $p < 0.05$), despite comparable BMD [13].

Bone status in our EDS cases

The two reported fractured patients are good examples of different type of bone findings that we observe in EDS in our clinical practice. In fact, it is relatively frequent to find patients with normal or slightly decreased BMD who are already fractured at diagnosis or may fracture during follow-up (patient n.1). On the other hand, it is also possible to find patients with clinically relevant densitometric osteoporosis and radiological vertebral fractures or development of new fractures during follow-up (patient n.2).

The heterogeneity of bone presentation can be hypothesized to be due to a molecular defect in collagen which may cause different bone phenotypes in EDS ranging from defects prevalently in bone quality (patients n.1), as often found in many forms of secondary osteoporosis [14, 15], to those patterns of classic bone loss (patient n. 2) which resemble those observed in OI [23–25]. Interestingly, in common with OI, EDS patients show the precocity of bone damage in which hypogonadism does not seem to play a key role [24]. Some EDS patients may also have variable degrees of hypovitaminosis D, which may contribute to their skeletal impairment. Finally, bone markers do not seem consistently affected in our patients in whom they may be either increased or normal with a picture not dissimilar to that observed in OI [31, 32], supporting the hypothesis of a variable pathogenesis of bone damage in EDS.

Proposed structured approach to bone evaluation in EDS

Paradoxically, bone complications in EDS have been clinically neglected so far, despite several reports suggesting the existence of multiple patterns of skeletal fragility in affected patients. As a consequence, since no guidelines in this specific setting are available, the best personalized diagnostic and therapeutic approach to bone complications in EDS is totally unknown and left to the expertise of the single clinicians.

In our practice, bone complications are common in EDS affecting more than 1/3 of this population. In fact, a significant step forward in the better understanding of bone involvement in EDS has been represented, as in other endocrine and not endocrine diseases [14, 33, 34] by the widespread application to our EDS patients of the morphometric approach in the assessment of vertebral fractures. This was adopted after our seminal study reporting increased prevalence of morphometric vertebral fractures in EDS vs controls [14]. In fact, the morphometric approach is now well accepted as the gold standard of clinical evaluation of skeletal fragility in post-menopausal and secondary osteoporosis [14, 15, 35], since clinical or indirect diagnosis of these fractures based on questionnaires may heavily underestimate them [36, 37].

Structured diagnostic and therapeutic approach to bone complications in EDS is in our opinion a significant unmet need. Our diagnostic approach includes: measurement of BMD by DXA, assessment of vitamin D status and morphometric vertebral fractures evaluation. In particular, this latter tool is crucial, also due to the presence of fractured EDS patients with marginal DXA BMD alterations. Measurement of bone markers and of TBS can be useful in these latter patients with fractures and normal BMD (Fig. 1).

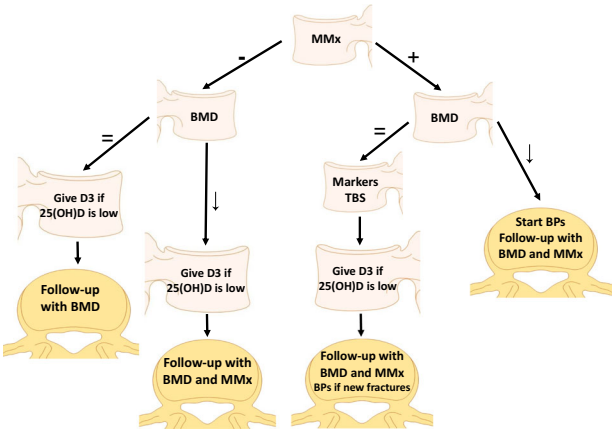

Fig. 1 Proposed approach to the managment of bone complications in EDS patients. MMx vertebral morphometry on spine Rx; BPs bisphosphonate; = normal BMD; down arrow osteopenia or osteoporosis

Our treatment approach to skeletal complications in EDS is based on integration of vitamin D in the frequent cases in which hypovitaminosis D is found. This approach may be sufficient in patients with BMD alterations but without fractures.

Bisphosphonate administration, for example, with neridronate, may be suggested in patients who, already at young age, develop bone loss and fractures as already reported in OI, despite a not significant increase in bone resorption markers [32] (Fig. 1).

Finally, in those patients bearing morphometric fractures (particularly if isolated and mild) despite persistently normal or only modestly impaired bone mass, our treatment is initially based on the correction of modifiable risk factors (smoke, low calcium intake) and vitamin D deficiency. Strict (yearly) densitometric and morphometric follow-up is applied. Antiresorptive treatment may be commenced during the follow-up if a marked bone skeletal damage is observed and in particular if a morphometric vertebral fracture develops.

Conclusions

Based on our experience, we should proactively screen all EDS patients since they are at high risk for low bone mass and fractures. Bone evaluation should also include vertebral morphometry since in some patients, despite a BMD not always severely impaired, an increased fracture risk is observed. In this type of patients, it is also extremely important to correct potential risk factors for bone health. Due to the several existing forms of EDS and of the variable bone phenotype in EDS, large cohort prevalence and incidence studies are needed on morphometric and clinical fractures in order to make available to clinicians evidence-based guidelines for the treatment of skeletal complications in EDS.

Compliance with ethical standards

Conflict of interest A.G. is consultant for Abiogen. The remaining authors declare that they have no conflict of interest.

Ethical approval This article does not contain any experimental studies with human participants performed by any of the authors. Data collected from routine evaluation of two patients are reported.

Informed consent Informed consent was obtained from the two patients whose routine data were reported in the manuscript.

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