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Bone impairment in oxalosis: An ultrastructural bone analysis☆

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ABSTRACT

Deposition of calcium oxalate crystals in the kidney and bone is a hallmark of systemic oxalosis. Since the bone compartment can store massive amounts of oxalate, patients present with recurrent low-trauma fractures, bone deformations, severe bone pains and specific oxalate osteopathy on plain X-ray. Bone biopsy from the iliac crest displays specific features such as oxalate crystals surrounded by a granulomatous reaction due to an invasion of bone surface by macrophages. We present data obtained in 10 samples from 8 patients with oxalosis (16–68 years) who underwent iliac crest bone biopsy and bone quality analysis using modern methods (microradiography, microindentation, Fourier Transform InfraRed Microspectroscopy, transmission electron microscopy) in addition to histomorphometry. Disseminated calcium oxalate deposits (whewellite) were found in the bone marrow space (with a granulomatous reaction) but not in the bone matrix. Calcium oxalate deposits were totally surrounded by macrophages and multinucleated giant cells, and a phagocytosis activity was sometimes observed. Very few calcium oxalate crystals were directly in close contact with the mineral substance of the bone. Bone mineralization was not modified by the presence of calcium oxalate even in close vicinity. Bone quality analysis also revealed a harder bone than normal, perhaps in relationship with decreased carbonate content in the mineral. This increase in bone hardness could explain a more "brittle" bone. In patients with oxalosis, the formation and growth of calcium oxalate crystals in the bone appeared independent of apatite. The mechanisms leading to nucleation and growth of oxalate deposits are still unclear and deserve further studies.

1. Introduction

Calcium oxalate is a highly insoluble salt, corresponding to an end-product of the metabolism of carbohydrate, vitamin C and some amino-acids [1]; it is excreted almost entirely by the kidney [2]. Primary hyperoxalurias (PHs) are a group of orphan autosomal recessive diseases inducing an overproduction of oxalate from the liver, leading first to renal deposition of insoluble oxalate crystals leading to almost constant nephrocalcinosis, as recently summarized in a thorough review on the topic [2]. A global deposition of insoluble oxalate crystals (also known as systemic oxalosis) occurs when renal function declines. End-stage renal disease (ESRD) can occur anytime between the first months and the sixth decade of life, however before 25 years in 50% of cases [3]. Oxalate crystals can be found in kidneys, urinary tract, bones, vessels, heart, nerves, joints, skin, bone marrow, soft tissues, liver and retina [3]. In the most severe cases of PH, namely PH1 (mutations in *the AGXT gene* encoding the peroxisomal alanine-glyoxylate aminotransferase enzyme), the overall prognosis has dramatically improved with the development of combined (synchronous or sequential) liver–kidney transplantation (CLKT); in less severe phenotypes of PH, such as PH2 (mutations in the *GRHPR* gene encoding the cytosolic glyoxylate reductase–hydroxypyruvate reductase), ESRD may also occur. In the other forms/types of PH, for example in PH3 (mutations in the *HOGA1* gene, encoding the mitochondrial 4-hydroxy-2-oxoglutarate aldolase), ESRD has not been described so far [4]. Hyperoxaluria may also be 'secondary' and then occurs as a result of excess dietary intake or the result of enteric hyperoxaluria such as in Crohn disease and bypass surgery; even though these forms may lead to ESRD [5], no specific data on bone impairment are available in this setting.

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The bone compartment can store massive amounts of oxalate [6,7], but the threshold of glomerular filtration rate (GFR) at which this occurs is debatable and might be as high as 30 to 45 mL/min per 1.73 m^2, i.e., CKD3 [3,8,9]. Patients with PH1 – and less frequently patients with PH2 – can present with severe impaired renal function, and thus systemic oxalosis and bone damage [2]. From a clinical point of view, they can experience severe bone pain, pathological fractures and bone deformations [10]. Although regarded as the reference standard for bone evaluation, iliac crest bone biopsy is an invasive procedure that cannot be recommended for routine follow-up [11]. If performed in patients with PH, oxalate crystals are isolated or grouped in clusters, forming star-like figures often surrounded by a granulomatous reaction due to invasion of bone surface by macrophages. Indeed the presence of deposits of calcium oxalate into the bone induces a specific granulomatous reaction since macrophages do not phagocytize crystals but appear to be involved in bone resorption [12–14].

Besides physiological calcifications (apatite in the bone, teeth, calcified cartilage), pathological deposits (apatite, calcium pyrophosphate, calcium oxalate) have been identified in human joints and numerous soft tissues (kidney, skin, muscles, blood vessels), but also in bone tissue, mainly in the bone marrow in the vicinity of the trabeculae from cancellous bone tissue. It should be emphasized that calcium oxalate deposits were exclusively reported as case reports in PH patients without global analysis in a substantial number of patients. Indeed scanning and transmission electron microscopy analyses have already been performed on calcium oxalate deposits in soft tissues, and X-ray diffraction studies have been essentially used to identify the nature of crystals in urinary calculi, but specific data in the bone are missing, since biophysical and ultrastructural methods are rarely used to study human bone lesions during oxalosis course [15–17].

The objective of the present study is therefore to present a single-center experience of bone biopsy and bone ultrastructural analysis in oxalosis. The main objective of the paper was to evaluate whether the presence of an 'atypical' calcium salt (i.e., calcium oxalate) close to the bone matrix would induce modifications of the quality of the 'physiological' bone mineral, i.e., the apatite crystals. Therefore, we present the data obtained in patients with oxalosis who underwent iliac crest biopsy allowing the assessment of bone quality using histology, microradiography, microindentation (microhardness of bone tissue), infrared microspectroscopy and transmission electron microscopy. The bone quality was evaluated close to oxalate deposits, and the interactions between calcium oxalate crystals, bone mineral substance and organic matrix were also assessed.

2. Patients and methods

The present study was carried out on 10 human iliac crest bone biopsies from 8 patients (3 females, 28 to 68 years old; 4 males, 16 to 52 years old). Eight of these samples (corresponding to 6 patients) were obtained before 1989 with a Bordier core needle (7.5 mm diameter); for these patients, clinical characteristics are not exhaustive but histomorphometry analysis was available in seven biopsies from 6 patients suffering from either primary or secondary oxalosis. As a marker of bone mass, cancellous bone volume (Cn BV/TV) was increased in 4, normal in 1 and decreased in 2 samples. As a marker of bone remodeling/bone resorption, cancellous eroded surface (Cn ES/TS) was increased in 6 samples and normal in 1 sample. As a marker of mineralization, cancellous osteoid surface (Cn OS/TS) was increased in 6 samples and normal in 1 sample, while cancellous osteoid thickness (Cn OTh) was increased in 5 and decreased in 2 samples. Eventually, as a marker of bone volume, cancellous osteoid volume (Cn OV/TV) was increased in 5 and normal in 2 samples. These semi-quantitative analyses were performed in comparison to historical age- and gender-matched controls, with a 5%-variation to controls considered significant.

Two bone biopsies were performed in 2014 to improve clinical decision making; they were obtained in one patient with PH1 (after CLKT) and one patient with PH2 (undergoing hemodialysis without a past of CLKT); they were obtained with a Jamshidi core needle (3 times thinner than Bordier's needle), therefore preventing us to perform complete histomorphometry analysis; characteristics of these two patients are summarized in Table 1. The main illustrations of this manuscript are referred to these last two bone samples from patients with PH1 and PH2.

Results were compared to control subjects (iliac bone biopsies from 2 males and 3 females, age range 21–45 years) that were measured in the same conditions than the bone from patients with oxalosis, as previously published [18]. Since the first part of the manuscript corresponds to a retrospective case series and the second part of the manuscript corresponds to biopsies performed to improve clinical decision-making as recommended by international guidelines [19], it did not require any local ethics committee evaluation.

For sample preparation, undecalcified iliac bone samples were fixed in 70° ethanol, dehydrated in absolute ethanol and embedded in methyl methacrylate, without decalcification. For histology (normal or polarized light), 8 μm-thick sections were cut from embedded samples at very low speed using a Leica Polycut S (Düsseldorf, Germany) microtome equipped with a tungsten carbide knife. Microradiography was assessed on 100 μm-thick sections [20,21]. Microhardness was measured on the surfaced blocks of the bone [21]. FTIRM was performed

Table 1
Characteristics of the two recent PH1 and PH2 patients with bone biopsies.

	PH1 patient	PH2 patient
Age at end stage renal disease (i.e., beginning of hemodialysis) (years)	9	37
Age at transplantation (years)	14	Not performed yet
Requirement of hemodialysis after transplantation	No	Not applicable
Age at bone biopsy (years)	16	40
Genetic analysis	*AGXT* gene, c.33_34insC/p.Gly170Arg	*GRHPR* gene, exon 7, p.Ser209Phe
Renal function at the time of bone biopsy	2 years after combined kidney/liver transplantation, GFR 25 mL/min/1.73 m^2 (iohexol clearance)	Hemodialysis
Urine oxalate:creatinine ratio at the time of bone biopsy (μmol/μmol, normal values < 0.08)	0.11	Not available (anuria)
Plasma oxalate:creatinine ratio at the time of bone biopsy (μmol/μmol, normal values < 0.09)	0.094	Not determined
Clinical signs of bone involvement at the time of bone biopsy	No	Yes, diffuse bone pains
Bone symptoms during follow-up	Fractures Lower limb deformations requiring surgery	Diffuse bone pain Diffuse spinal osteocondensation Areas of bone resorption on X-rays (sacro-iliac joint, hands)