Clinical Case Report

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Improvement of bone microarchitecture parameters after 12 months of treatment with asfotase alfa in adult patient with hypophosphatasia

Case report

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Abstract

Rationale: Hypophosphatasia is an inborn error of metabolism that can appear any time in life, mainly with bone manifestations due to low alkaline phosphatase activity. Asfotase alfa is a specific enzyme reposition treatment that has shown promising results in children; however, there are few reports about the outcomes in adult patients.

Patient concerns: A 36-year-old male presented with an early history of craniosynostosis, short stature, and multiple fractures since the age of 13 years—which needed numerous surgical corrections. He was admitted with a previous diagnosis of *osteogenesis imperfecta*, taking alendronate, calcium carbonate, cholecalciferol, and calcitriol. Bone mineral density was low (lumbar spine *Z*-score = −3.0 SD), with impairment of all parameters of high-resolution peripheral quantitative computed tomography (HR-pQCT). Kidney impairment was also observed with reduced creatinine clearance, nephrolithiasis, and nephrocalcinosis.

Diagnosis: Alkaline phosphatase was unexpectedly low (6 U/L, reference value: 30–120 U/L), with high serum vitamin B6 (260 mcg/L, reference value: 5.2–34.1). Genetic testing showed a homozygous missense mutation in *ALPL* gene c.443 C>T: p.Thr148Ile.

Intervention: Asfotase alfa was requested due to important bone deterioration, ambulatory disability, and kidney impairment. It was given subcutaneously 2 mg/kg per dose, 3 times a week, for 12 months before reassessment.

Outcomes: Bone mineral densities of the lumbar spine and whole body, besides almost all HR-pQCT microstructural parameters of the distal tibia, showed improvements and the patient was able to walk without assistant device. Kidney function did not further deteriorate.

Lessons: Hypophosphatasia should be considered as a differential diagnosis in young patients with multiple fractures and kidney impairment, since the use of antiresorptive drugs, calcium and vitamin D, commonly used to treat fractures, worsen its symptoms and prognosis. A 12-month asfotase alfa treatment improved bone density and structural parameters even in an adult patient with late diagnosis.

Abbreviations: *ALPL* = alkaline phosphatase, liver/bone/kidney, BMD = bone mineral density, CTX = C-terminal telopeptide of type I collagen, CV = coefficient of variation, HPP = hypophosphatasia, HR-pQCT = high-resolution peripheral quantitative computed tomography, MDRD = modification of diet in renal disease, P1NP = procollagen type I N-terminal propeptide, SD = standard deviation.

Keywords: alkaline phosphatase, bone, bone remodeling, fractures, hypophosphatasia, nephrolithiasis

1. Introduction

Hypophosphatasia (HPP) is an inborn error of metabolism with a birth prevalence of roughly 1:100,000.[1] It is considered an inherited bone metabolism disorder with mainly bone manifestations that can appear any time in life.[2] There is no specific mutation that promotes this disease; however, mutant alleles have a dominant-negative effect or a loss of effect upon the alkaline phosphatase *ALPL* gene.[3] The direct consequence of a malfunctioning enzyme is elevated concentration of its substrates; in this case: inorganic pyrophosphate, pyridoxal phosphate, and phosphoethanolamine.[4,5]

HPP has 6 clinical subtypes,[4] but 4 of them are more relevant: perinatal, infantile, juvenile, and adult forms. It is important to highlight that when symptoms appear or remain during adulthood, the diagnosis is more challenging due to its many possible differentials, such as *osteogenesis imperfecta*, rickets, osteoporosis, chondrocalcinosis, nephrocalcinosis, and nephrolithiasis.

Laboratory findings such as unexplainably low serum alkaline phosphatase activity, elevation of serum pyridoxal-5′-phosphate

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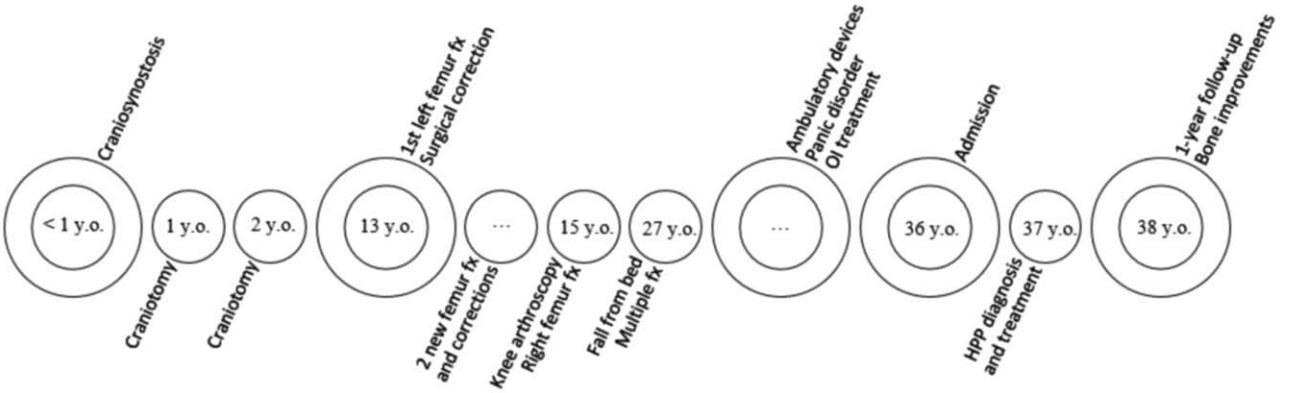

Figure 1. Patient timeline since birth. fx = fracture, HPP = hypophosphatasia, OI = *osteogenesis imperfecta*, Y.O. = years old.

(vitamin B6) or urinary phosphoethanolamine may support the clinical diagnosis.[6] *ALPL* gene mutations may also be investigated for confirmation; to date, more than 350 mutations causing hypophosphatasia have been described.[7]

Wrong or late diagnosis is noxious, since treating for differential diagnosis can exacerbate symptoms. Most bone metabolism disorders respond to calcium plus calcitriol supplementation; and bisphosphonates treat conditions like osteoporosis and *osteogenesis imperfect*; however, these same drugs worsen symptoms and prognosis in hypophosphatasia.[8]

A new specific treatment, asfotase alfa, a human recombinant tissue-nonspecific alkaline phosphatase with a bone-targeting domain, was approved in 2015 in the United States, Japan, and European Union for pediatric patients with hypophosphatasia, regardless of their current age during treatment.[9,10] Asfotase alfa has shown promising results in children,[11] however, there are few reports about the outcomes of the specific treatment using enzyme reposition in adults.[12] Hence, this report aims to describe a case of an adult patient with hypophosphatasia, presenting mainly with fractures, and the therapeutic outcomes 12 months after enzyme reposition.

2. Case report

CC, a 36-year-old male, presented with an early history of craniosynostosis, requiring craniotomy at 12 and 24 months of age. He had always had short stature, with a final height of 130.5 cm. During childhood, there was no history of premature tooth loss; nonetheless, a history of multiple fractures began 23 years prior to admission, after falling from his own height, which led to a left femur neck fracture that required osteosynthesis. Afterward, he fractured the same site twice, needing correction of bone nonunion. Two years later, when he was 15 years old, a knee arthroscopy meant to investigate meniscal damage led to a distal right femur fracture. Twelve years after the last episode, at age 27, a fall from bed caused fractures of the right humerus, right pelvis, and right femur neck, requiring internal fixation of humerus and hip arthroplasty. Since then, his mobility reduced, and he became unable to independently dress himself, go to work and even move around the house. This brief history is depicted in a simplified timeline (Fig. 1).

At the time of admission, the patient had a diagnosis of *osteogenesis imperfecta*, treating with calcium carbonate, cholecalciferol, calcitriol, and taking psychotropic drugs for panic disorder. He had a history of using alendronate 70 mg weekly for eight years until one year prior to admission.

In order to reassess and further evaluate his bone metabolism, a laboratory workup was performed. No abnormalities in serum biochemical and hormone markers were seen. Bone turnover markers were also within normal range, but alkaline phosphatase was unexpectedly low. Additionally, renal function was altered: creatinine clearance estimated by Modification of Diet in Renal Disease (MDRD) equation[13] showed a stage III chronic kidney disease (Table 1).

Table 1

Initial and follow-up laboratory evaluation.

Biochemical and hormone markers	Admission	12 months follow-up	Reference values
Total calcium, mg/dL	10.2	9.8	8.5–10.2
Ionic calcium, mg/dL	5.24	5.17	4.60–5.30
24 hs urine calcium (mg/kg/24-hour volume)	1.5	0.12	2–4
Phosphorus, mg/dL	4.3	5.1	2.7–4.5
Parathyroid hormone, pg/mL	45	79	15–65
25-hydroxyvitamin D, ng/mL	30	29	>30
Bone turnover markers	**Admission**	**12 months follow-up**	**Reference values**
C-terminal telopeptide of type I collagen (CTX), ng/mL	0.45	0.92	<0.70
Procollagen type I N-terminal propeptide (P1NP), ng/mL	58.4	106.3	13.9–85.5
Alkaline phosphatase, U/L	6	2749	30–120
Kidney function	**Admission**	**12 months follow-up**	**Reference values**
Creatinine, serum, mg/dL	1.46	1.51	0.70–1.20
Creatinine clearance, mL/min/1.73 m^{2*}	58.2	55.5	>90

CTX = C-terminal telopeptide of type I collagen, P1NP = procollagen type I N-terminal propeptide.
* Creatinine clearance estimated by MDRD equation.[13]

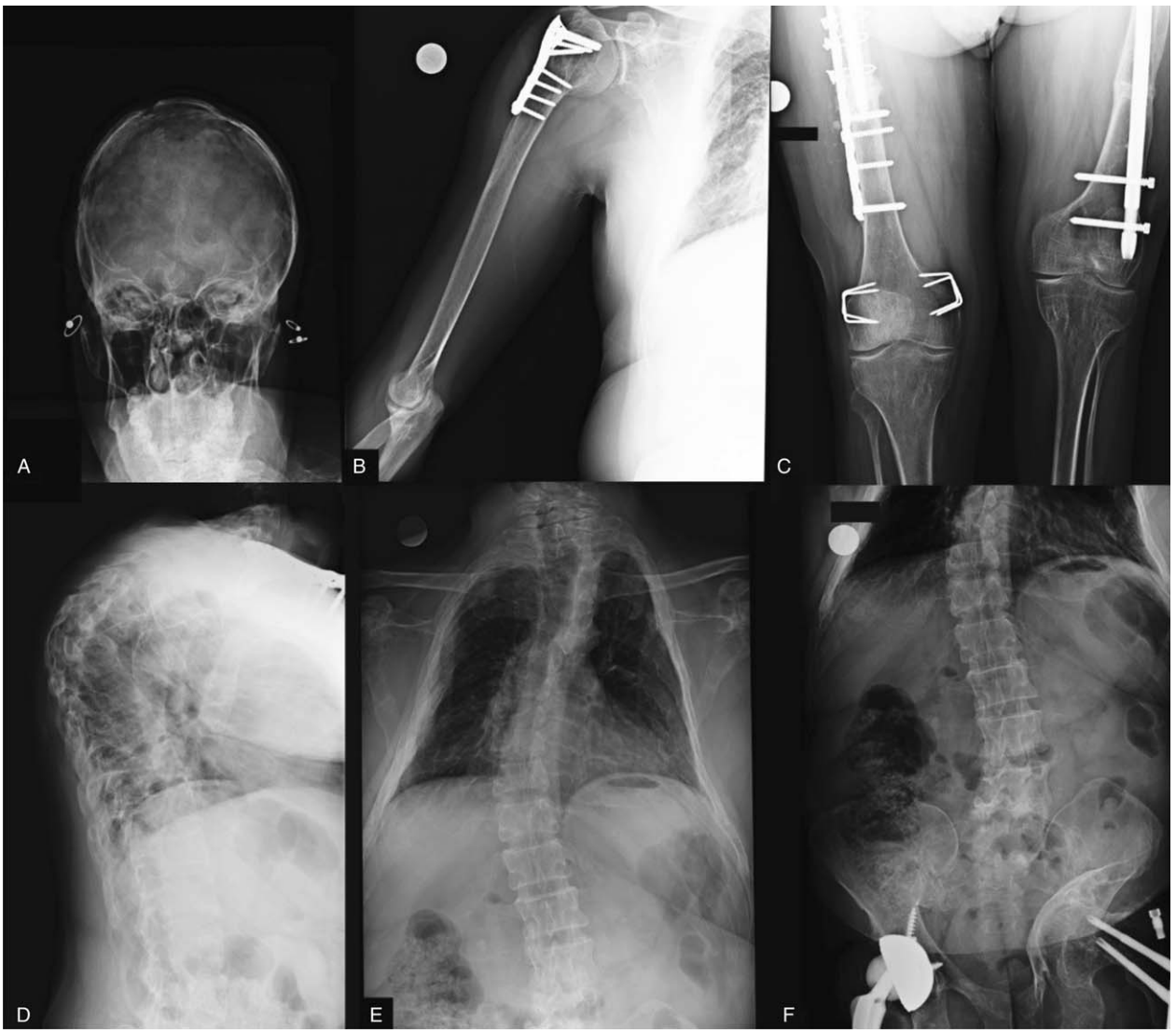

Figure 2. Conventional radiographs. (A) Skull, with signs of craniotomy; (B) right humerus, with proximal screws and plates fixation; (C) femur and knees: bone malunion/nonunion bilaterally despite screws and plates fixations; (D, E) thoracolombar spine [(D) lateral; (E and F): anterior–posterior scans], showing kyphoscoliosis and right total hip arthroplasty.

Common radiographs were performed to evaluate the multiple fractures and orthopedic procedures: evident kyphoscoliosis and vestiges of craniotomy were observed (Fig. 2).

Bone densitometry was determined by dual energy x-ray absorptiometry (DXA, Hologic QDR 4500A, Bedford, MA) and showed low bone mineral density (BMD) for chronological age, with lumbar spine *Z*-score of −3.0 SD and a 1/3 distal left radius *Z*-score of −4.4 SD. Hip and whole body BMD were analyzed, but not initially considered for diagnostic purposes due to the presence of bilateral hip prosthesis, and internal fixations in right knee and humerus.

High-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, SCANCO Medical AG, Brüttisellen, Switzerland) was performed to analyze cortical and trabecular bone compartments, including volumetric density, structure parameters, and biomechanical bone strength, and it showed an impairment of them all (Table 2).

Kidney ultrasound showed chronic parenchymatous nephropathy with nephocalcinosis, bilateral nonobstructive nephrolithiasis with parietal calcifications, and stones ranging from 0.6 to 0.8 cm, and cysts on the right kidney.

Continuous evaluation proceeded: serum vitamin B6 260 mcg/L (reference value: 5.2–34.1), alkaline phosphatase persisted low: 12 U/L (reference value: 30–120). Genetic testing (Centro de Genomas, Sao Paulo, SP, Brazil) showed a homozygous missense mutation in *ALPL* gene c.443 C>T: p.Thr148Ile, which has already been identified in other patients with hypophosphatasia.[14] A probably benign heterozygous mutation was also found in the *ALPL* gene: c.1190-266_1190-251del16 (rs 145806416). The only available relative who accepted to be tested was his mother, who presented the same missense mutation in *ALPL* gene c.443 C>T: p.Thr148Ile, in heterozygosity.

After confirming the diagnosis, specific treatment with enzyme reposition (asfotase alfa—2 mg/kg/dose, 3 times a week) was requested to slow disease progression,[15] aiming for an improvement especially in bone and motor functions.[16] Twelve months after treatment, a new bone mineral densitometry was performed and showed significant improvements in lumbar spine

Table 2

Volumetric bone density, structure and strength parameters obtained by high-resolution peripheral quantitative computed tomography (HR-pQCT), compared at admission, 12 months follow-up, and with healthy male controls 30–39 years old.

	Distal Radius				Distal tibia			
HR-pQCT	Admission	12 months Follow-up	Difference (%)	Male controls median (IQR)	Admission	12 months follow-up	Difference (%)	Male controls median (IQR)
Total volumetric BMD, mg HA/cm^3	219	218	−1.0 (−0.4)	350 (304–389)	155	167	12 (7.6)	323 (299–367)
Trabecular volumetric BMD, mg HA/cm^3	105.3	104.5	−0.8 (−0.7)	211 (178–213)	115	123	8 (6.9)	198 (175–222)
Cortical volumetric BMD, mg HA/cm^3	836	835	−1.0 (−0.1)	890 (846–910)	599	636	37 (6.2)	916 (895–930)
Trabecular number, 1/mm	1.54	1.54	0 (0)	2.24 (2.03–2.37)	1.07	1.07	0 (0)	2.10 (1.87–224)
Trabecular thickness, mm	0.057	0.057	0 (0)	0.079 (0.071–0.086)	0.090	0.096	0.006 (6.6)	0.078 (0.068–0.095)
Trabecular bone separation, mm	0.591	0.593	0.002 (0.3)	0.370 (0.343–0.418)	0.847	0.838	−0.008 (−1.0)	0.396 (0.366–0.453)
Cortical thickness, mm	0.56	0.54	−0.02 (−3.6)	0.86 (0.73–1.00)	0.28	0.34	0.06 (22.4)	1.39 (1.19–1.51)
Stiffness, kN/mm*	61.128	59.345	−1.783 (−2.9)	118.709 (72.538–180.046)	90.462	102.028	11.566 (13)	306.176 (240.042–427.341)

BMD = bone mineral density.
* Estimated by finite element analysis.

and whole body BMDs (Fig. 3), since the least significant change in our laboratory is 0.023 g/cm^2 at lumbar spine and 0.022 g/cm^2 for the whole body scan. A bone microarchitecture evaluation was also repeated (Table 2). There was an improvement in almost every parameter in the distal tibia, with exception to trabeculae number and trabecular bone separation. No significant changes were noted in the distal radius parameters, considering the coefficient of variation (CV) of HR-pQCT from our laboratory (distal tibia: density CV = 0.25%–1.16%, structure CV = 0.78%–6.35%; distal radius: density CV = 0.93%–1.41%, structure CV = 0.78%–6.35%) (Table 2).[17]

3. Discussion

It is important to draw attention to hypophosphatasia as a differential diagnosis of multiple fractures as the main clinical manifestation in an adult patient since childhood. *Osteogenesis imperfecta*, which is another relevant differential diagnosis, was ruled out because the patient also presented with craniosynostosis and kidney impairment. Moreover, the remarkably low levels of alkaline phosphatase observed pointed toward hypophosphatasia. However, in this patient, the low levels of this enzyme could be due to bisphosphonate chronic use, since this drug inhibits bone turnover.[18] The fact that other bone turnover markers, as C-terminal telopeptide of type I collagen (CTX) and procollagen type I N-terminal propeptide (P1NP), were at normal ranges in addition to the finding of elevated vitamin B6 strongly suggested hypophosphatasia as the main diagnosis,[2,6,19,20] which was confirmed by genetic testing.

Hence, bisphosphonates, calcium, and vitamin D supplementation were discontinued, since these drugs worsen HPP: without proper alkaline phosphatase function, there can be no calcium and phosphorus mineralization. Therefore, providing more calcium and vitamin D leads to its build up specially in the kidneys, worsening nephrocalcinosis and nephrolithiasis.[4] Vitamin D supplementation may also lead to hypercalcemia and increase calciuria.[21] Regarding bisphosphonates, they worsen bone mineralization, which is already compromised, leading to even more severe bone fragility.[22,23] Furthermore, tissue nonspecific alkaline phosphatase can be inhibited by N-containing bisphosphonates[24] and may increase atypical fractures even in carriers of heterozygous mutations.[25] Indeed, hypophosphatasia should be considered as a differential diagnosis of atypical fractures caused by bone mineralization deterioration due to bisphosphonate chronic use.

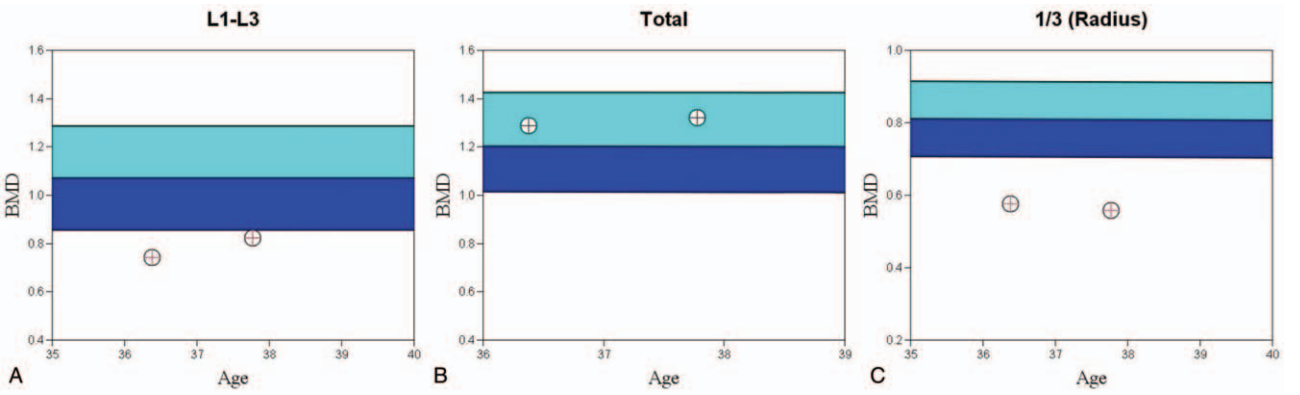

Figure 3. Graphs showing temporal evolution of bone mineral density. (A) Lumbar spine BMD (L1–L3): 0.742 to 0.822 g/cm^2 (0.08 g/cm^2, or 10.8% increase). (B) Whole body BMD: 1.287 to 1.320 g/cm^2 (0.033 g/cm^2, or 2.5% increase). (C) Left proximal third distal radius BMD: 0.575 to 0.559 g/cm^2 (−0.017 g/cm^2 or 2.9% decrease). BMD = bone mineral density.

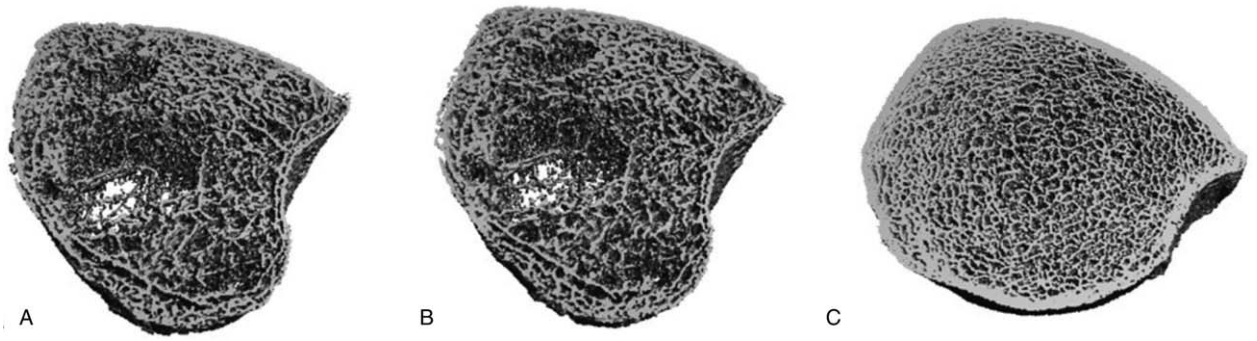

Figure 4. High-resolution peripheral quantitative computed tomography images of distal tibia. (B) patient at admission. (B) patient after treatment for 12 months (B) age- and gender-matched control.

Considering the patient's important ambulatory disability due to multiple fractures, which could worsen with disease progression, treatment with enzyme reposition was recommended based on literature.[6,12] Enzyme replacement is achieved with asfotase alfa, a human recombinant tissue-nonspecific alkaline phosphatase. Its modifications from the original molecule include an IgG Fc domain, and a repetitive C-terminal extension of 10 Asp residues, which was designed to have a high affinity for hydroxyapatite crystals (e.g., a bone-targeting domain). Once in the bone, asfotase alfa assembles into an enzymatic active tetramer capable of hydrolysing inorganic pyrophosphate, the same role human tissue-nonspecific alkaline phosphatase plays in the bone. It is hypothesized that asfotase alfa, as alkaline phosphatase, regulates bone mineralization by regulating inorganic pyrophosphate concentrations, which are both a substrate for hydroxyapatite crystal formation and an inhibitor of its nucleation and growth.[26]

An improvement of areal/volumetric bone mineral density, microarchitecture parameters and biomechanical proprieties at distal tibia and stabilization of these parameters at distal radius were observed after 12 months of treatment (Table 2 and Fig. 4), which was also associated with a better quality of life, since the patient was able to walk without assistant devices.

In conclusion, unexpectedly or persistently low values of alkaline phosphatase with bone and systemic manifestations should draw attention to hypophosphatasia as an important diagnosis. This disease has specific treatment and the late diagnosis and wrong treatment, mainly with antiresorptive drugs and calcium plus vitamin D supplementation, commonly used to treat many of HPP's differentials diagnoses, could increase morbidity and lead to mortality. Additionally, considering its difficult diagnosis and complex management, patients with HPP should be referred to tertiary or academic medical centers, which can provide specialists in metabolic bone disorders and easier access to newer treatments.[20]

Recently, asfotase alfa treatment showed promising results in children, and this case report, as other researches,[12,27] boost evidence that it may be successful also in adult patients with hypophosphatasia.

4. Patient consent statement

Informed written consent was obtained from the patient for publication of this case report and accompanying images.

Author contributions

Conceptualization: Rosa Maria Rodrigues Pereira.
Funding acquisition: Rosa Maria Rodrigues Pereira.
Investigation: Thiago Quadrante Freitas, Andre Silva Franco, Rosa Maria Rodrigues Pereira.
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Supervision: Rosa Maria Rodrigues Pereira.
Writing – original draft: Thiago Quadrante Freitas, Andre Silva Franco, Rosa Maria Rodrigues Pereira.
Writing – review & editing: Thiago Quadrante Freitas, Andre Silva Franco, Rosa Maria Rodrigues Pereira.

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