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The Challenge of Hypophosphatasia Diagnosis in Patients with Fibromyalgia

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Highlights of the Study

- 3.2% of fibromyalgia patients show persistently decreased levels of total alkaline phosphatase.
- Low levels of total alkaline phosphatase in fibromyalgia patients seem to be mainly related to comorbidities.
- When evaluating hypophosphatasia in patients with fibromyalgia a genetic study is also recommended.

Keywords
Hypophosphatasia · Fibromyalgia · Alkaline phosphatase · Screening

Abstract

Objective: Recent reports suggest that mild forms of hypophosphatasia (HPP) may be misdiagnosed as fibromyalgia (FM), thus exposing patients to potential complications (e.g., fractures). It seems reasonable to determine alkaline phosphatase (ALP) levels in all patients diagnosed with FM. We aimed to study this clinical recommendation by assessing the prevalence of HPP in a cohort of patients diagnosed with FM in a specialized unit. ***Subjects and Methods:*** This retrospective study (2014–2021) included 713 patients with FM and previous determinations of ALP levels from a Multidisciplinary Fibromyalgia Unit of a Rheumatology Department. Medical records (ALP levels, history of fractures, radiologic studies, pharmacological treatment, and comorbidities) were reviewed. Patients with at least two ALP determinations under normal values were further evaluated with a study of bone metabolic parameters, ALP substrate [PLP: pyridoxal-5′-phosphate] and genetic testing for alkaline phosphatase, liver/bone/kidney (*ALPL*) pathogenic variants. ***Results:*** 16 (3.2%) FM patients (all women with a median age of 50 years) showed low ALP levels. Notably, 5 patients (31.3%) also showed elevated PLP levels, suggesting HPP. However,

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none presented pathogenic *ALPL* variants in the genetic study. Other associated conditions, such as subclinical hypothyroidism and the use of dietary supplements/multivitamins, were observed in some of these patients. ***Conclusion:*** In the present cohort, 3.2% presented persistently decreased ALP levels. The increased *ALPL* substrate, PLP, observed in one third of these subjects, together with the negative genetic study in all these subjects, indicate the need to better identify the subjects with FM that may have mild forms of HPP.

Introduction

Hypophosphatasia (HPP) is a rare-inherited disease caused by diminished activity of tissue-nonspecific alkaline phosphatase (TNSALP), primarily attributed to pathogenic or likely pathogenic variants (P/LP) in the *ALPL* gene. This deficiency results in impaired bone mineralization, leading to a diverse array of clinical manifestations affecting musculoskeletal, neurological, respiratory, renal, and dental systems, among others, with a broad spectrum of severity from neonatal lethality to paucisymptomatic presentations. The wide expression of ALP across various tissues, including bone, liver, and kidney, coupled with variable modes of inheritance, contributes to the clinical heterogeneity observed in HPP [1, 2].

Severe forms of HPP are typically associated with autosomal recessive transmission and lower ALP levels and can even be detected during the prenatal period due to occurrence of polyhydramnios, skeletal abnormalities, fractures, and pulmonary hypoplasia. Conversely, milder cases may evade detection until adulthood and are often only identified when active [2, 3]. Adult HPP is mostly associated with chronic pain syndromes characterized by musculoskeletal pain, muscle weakness and fatigue, and can also present with fragility stress fractures, chondrocalcinosis and calcific periarthritis [4]. Given its diverse clinical presentations, HPP can be overlooked as more common conditions such as osteoporosis (OP) [5], osteoarthritis (OA) [6], and, according to some recent reports, fibromyalgia (FM) [2, 7, 8]. This misdiagnosis may lead to diagnostic delay, thus conferring a high burden disease in adulthood and worsening symptoms due to inadequate treatment [8, 9].

HPP and FM may share symptoms such as chronic musculoskeletal pain, muscle weakness, fatigue, cognitive dysfunction, and mood disturbances [7]. Therefore, it might be advisable that individuals diagnosed with FM undergo comprehensive evaluation for potential underlying HPP. This assessment should combine a clinical exploration of manifestations suggestive of HPP (such as a history of premature tooth loss, fragility stress fractures, or calcific periarthritis) and further complementary tests in subjects with unexplained low ALP levels. Indeed, recent reports have suggested the possible misdiagnosis of FM in patients with adult forms of HPP, thus suggesting a routine measurement of ALP levels in these patients.

For this reason, our study aimed to explore the misdiagnosis of patients with HPP as FM. We designed an investigative approach involving screening for low ALP levels and conducting additional tests, including measurement of ALP substrates such as pyridoxal-5′-phosphate (PLP) and genetic analysis of the *ALPL* gene in subjects with decreased ALP levels. Early identification of HPP in this population may have important therapeutic implications and underscores the importance of tailored diagnostic strategies to optimize patient outcomes.

Subjects and Methods

We conducted a retrospective chart review encompassing all adult patients (aged >18 years) diagnosed with FM between 2014 and 2021 in a Multidisciplinary Fibromyalgia Unit of a Rheumatology Department, according to the 1990 and 2010 American College of Rheumatology (ACR) classification criteria. In this unit, these criteria were used in the entire cohort of patients. As part of our clinical practice, after the initial evaluation of a patient in the FM Unit, they may either be enrolled in our follow-up protocol for multidisciplinary treatment or discharged. In the patients included in the protocol, blood analysis in fasting state was performed which included alkaline phosphatase (ALP), among many other parameters. Patients who did not fulfill the inclusion criteria established by the FM unit protocol – irrespective of the availability of prior ALP determinations – were referred either to their primary care provider or to their designated regional rheumatologist. As a result, data from these individuals were not available for evaluation in the present study.

The data of all the patients with a confirmed diagnosis of FM who met the protocol-defined inclusion criteria for treatment at the FM unit were retrospectively analyzed. Thus, patients with at least two ALP determinations below

the reference range established by our institution (46–116 U/L) and who provided consent to undergo further bone metabolism evaluation, including imaging studies, laboratory testing, and genetic analysis were included in the study. The exclusion criteria for the study were patients who did not meet FM criteria; those with FM who did not meet the protocol-defined inclusion criteria for treatment within the FM Unit; patients who, despite showing two or more low determinations of ALP did not wish to undergo the additional metabolic study (blood test, imaging, and genetic test); or patients with FM with no ALP determination or with only one determination and inability to undergo a second analysis due to loss to follow-up.

The number of consecutive low ALP levels was recorded. We reviewed clinical, radiological, and laboratory data of the clinical reports of these patients, including age, gender, history of fractures, calcific tendonitis, prior OP diagnosis, DXA results (including the T-score values for bone mineral density assessment of lumbar spine and femoral neck), antiresorptive treatment involving bisphosphonates (BPs) or other agents, glucocorticoid treatment, and comorbidities including chondrocalcinosis and thyroid disease, among others. Additionally, the analytical results of all subjects with repeated low ALP values who accepted to complete their bone metabolism study, including bone metabolic parameters, such as serum calcium and phosphate, biochemical markers of bone turnover (including serum bone alkaline phosphatase [BAP], procollagen type I amino-terminal propeptide [PINP], as a markers of bone formation, and the cross-linked C-terminal telopeptide of type I collagen [CTX], and the urinary N-terminal telopeptide of type I collagen [NTX] as a markers of bone resorption), parathyroid hormone, 25-OH vitamin D, and ALP substrate PLP [vitamin B6] (range 15.0–96 nmol/L) were retrospectively collected. All these determinations were performed between 8:00 and 10:00 a.m. after overnight fasting. Genetic testing for evaluating *ALPL* P/LP variants was performed in these patients using standard procedures of Sanger sequencing. Deletions or duplications of exons or the whole gene was done with the MLPA technique following the manufacturer's instructions (MRC Holland, Amsterdam, the Netherlands). Sanger sequencing and MLPA results were analyzed using the SeqPilot software (JSI Medical Systems GmbH, Germany). Interpretations of the variants obtained were classified following the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG) [10].

The data collected underwent analysis employing descriptive statistics. The study was approved by the Ethics Committee of the Hospital Clinic of Barcelona (HCB/2024/0142). Informed consent was obtained from all patients who underwent the complete bone metabolism study, including genetic testing.

Results

During the study period, 952 patients with suspected FM were referred to our FM Unit for multidisciplinary evaluation. Of these, FM was confirmed in 713 patients; 36 had at least one low ALP level determination, and 23 individuals (3.2%) exhibited low ALP levels in at least two measurements (<46 U/L) and 16/713 (2.2%) accepted to complete the bone metabolic study (genetic, imaging and blood tests). The remaining 7 patients did not wish to undergo the metabolic study and were lost during follow-up.

The clinical characteristics of the 16 subjects with at least two determinations of low ALP levels that accepted to complete the bone metabolism study are shown in Table 1. All these 16 patients were female, with a median age of 50 years (range 33–69 years). Regarding comorbidities, 5 patients (31.25%) had associated hypothyroidism, with 3 receiving hormone replacement therapy with levothyroxine, and all showing normal thyroid hormone levels; 5 patients (31.25%) had concomitant autoimmune diseases (1 patient with Behçet's disease, 1 with psoriasis, 1 with spondyloarthritis HLAB27+, and 2 with SLE and Sjögren); 1 patient (6.7%) was undergoing antiresorptive treatment for OP (with denosumab) and was included for presenting low ALP levels prior to initiating denosumab treatment, and 1 other patient (6.25%) was receiving low doses of glucocorticoids (5 mg prednisone/day) for systemic lupus erythematosus. None of the patients were receiving BP treatment. All the patients analyzed, except for one, had multiple previous X-rays, which included at least the knees, hands, pelvis, feet, and lumbar spine and also the radiographies of locations of painful areas the patient had experienced in the past. Calcific tendonitis or enthesitis was observed in 6 patients (37.5%), with the rotator cuff affected in 2 cases, the common elbow extensor tendon in 2 cases, the achilles tendon in 1 case and the gluteus tendon in 1 case; none showed radiographic or clinical signs of chondrocalcinosis. Furthermore, no patient reported dental alterations suggestive of HPP. Three patients (18.8%) had previous fractures: 1 at tibia and fibula, at the age of 9 years with high impact; another

Table 1. Clinical characteristics of the 16 patients diagnosed with FM with low serum ALP levels

	Age, years	Gender	Antiosteoporotic treatment	GC treatment at the moment of ALP determination	Kidney stones or nephrocalcinosis	Comorbidities	Fractures or pseudofractures	DXA	Previous OP diagnostic	Radiological findings
Case 1	60	Female	No	No	No	Hypothyroidism Psoriasis	Tibia and fibula high impact fracture at 9 years old	T-score (L: −0.8, FN: −1.3)	No	Calcific enthesitis (iliac crest and gluteus medius)
Case 2	44	Female	No	No	No	Behçet's disease Autoimmune thyroiditis with hypothyroidism episodes	No	T-score (L: 0.4, FN: −0.8)	No	Recurrent of both calcific tendinitis and calcific enthesitis
Case 3	69	Female	Denosumab	No	No	No	Lumbar vertebra (L1), pelvis, ribs, coccyx	T-score (L: −3.6, FN: −2.1)	Yes	Calcific tendinitis (gluteus medius)
Case 4	58	Female	No	Yes; 5 mg prednisone	No	SLE and Sjögren	No	T-score (L: −1.5, FN: −2.1)	No	Calcific enthesitis
Case 5	47	Female	No	No	No	Hypothyroidism	No	T-score (L: 0.1, FN: −0.5)	No	Calcific tendinitis (rotator cuff)
Case 6	51	Female	No	No	No	Hypothyroidism	No	T-score (L: −0.5, FN: −1.3)	No	Scoliosis; no other alterations in X-ray
Case 7	50	Female	No	No	No	No	No	T-score (L: 0.8, FN: −0.4)	No	No alterations in X-ray
Case 8	55	Female	No	No	No	Spondyloarthritis HLA B27+	Rib	T-score (L: −2.2, FN: −1.5)	No	No alterations in X-ray

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Table 1 (continued)

	Age, years	Gender	Antiosteoporotic treatment	GC treatment at the moment of ALP determination	Kidney stones or nephrocalcinosis	Comorbidities	Fractures or pseudofractures	DXA	Previous OP diagnostic	Radiological findings
Case 9	44	Female	No	No	No	No	No	T-score (L: −1.8, FN: −1.8)	No	No alterations in X-ray
Case 10	57	Female	No	No	No	No	No	T-score (L: −0.8, FN: −0.7)	No	No alterations in X-ray
Case 11	49	Female	No	No	No	No	No	T-score (L: −2.7, FN: −2.1)	Yes	No alterations in X-ray
Case 12	33	Female	No	No	No	Hypothyroidism	No	T-score (L: 0.3, FN: −0.1)	No	No alterations in X-ray
Case 13	49	Female	No	No	No	Follicular lymphoma	No	T-score (L: 0.9, FN: −0.2)	No	Pectus excavatum
Case 14	38	Female	No	No	No	No	No	T-score (L: 0.4, FN: 2.0)	No	Tibial exostosis
Case 15	40	Female	No	No	No	SLE	No	No	No	No X-ray
Case 16	57	Female	No	No	No	No	No	T-score (L: 0.1, FN: −0.5)	No	Calcific tendinitis (cuff rotator)

Radiological findings include Rickets-like changes (by X-ray), deformities, chondrocalcinosis, calcified enthesis or ectopic calcification, calcific periarthritis. SLE, systemic lupus erythematosus; DXA, dual-energy X-ray absorptiometry; L, lumbar; FN, femoral neck; GC, glucocorticoids.

Table 2. Bone metabolic parameters of the 16 patients diagnosed with FM with low serum ALP levels

	Age, years	Gender	Total ALP	BAP	Ca	P	PINP	NTX	CTX	ALP/PINP ratio	25-OH vitamin D	PTH	Vitamin B6	Genetic *ALPL* study
Case 1	60	F	22	5.2	9.8	3.4	22	28	0.17	1	6	57	28.9	Negative
Case 2	44	F	36	9.7	9.2	3.5	31.2	16	0.14	1.153	21	–	62.8	Negative
Case 3	69	F	32	5.4	9.4	3.8	8.2	8	0.04	3.90	48.6	71	50.6	Negative
Case 4	58	F	41	10.3	9.6	3.6	37.3	60	0.41	1.099	21.8	59	23.5	Negative
Case 5	47	F	37	10.6	9.1	3.9	35.8	25	0.10	1.003	11.2	50	121.8	Negative
Case 6	51	F	35	–	9.8	3.5	–	–	–		16	79.3	72.3	Negative
Case 7	50	F	32	6.0	9.5	3.4	26.5	26	0.18	1.207	43.5	–	115.6	Negative
Case 8	55	F	40	13.4	9.4	4.2	51.3	62	0.43	0.779	37.3	47	311.8	Negative
Case 9	44	F	40.2	7.7	8.9	3.8	50.6	45	0.51	0.79S4	15.2	106	81.5	Negative
Case 10	57	F	39	10.2	9.8	3.8	45.4	43	0.32	0.859	121.7	–	188.6	Negative
Case 11	49	F	44	14.6	9.2	3.5	77.4	95	0.52	0.568	40.8	73	117	Negative
Case 12	33	F	45	6.4	9.6	3.3	40.4	32	0.30	1.113	23.3	51	67	Negative
Case 13	49	F	43	9.7	9	2.8	38.6	39	0.38	1.113	58.9	79	55.5	Negative
Case 14	38	F	45	7.7	8.8	3.4	36.5	33	0.17	1.232	41.6	35	37.3	Negative
Case 15	40	F	32	–	9.2	3.7	50.6	16	–	0.632	39.3	59	36.8	Negative
Case 16	57	F	31	9.7	9.3	3.5	24.9	36	0.10	1.244	15.9	84	21.6	Negative

Reference values: ALP, 46–116 U/L; BAP, 2.0–20.0 ng/mL; Ca, 8.5–10.5 mg/dL; P, 2.3–4.3 mg/dL; PINP, <55.0 ng/mL; NTX, <75 nMol/mMol; CTX, 0.02–0.58 ng/mL; 25OH vitamin D, >20 ng/mL (desirable value); PTH, 18–80 pg/mL; vitamin B6, 15–96 nmol/L. F, female; total ALP, total alkaline phosphatase; BAP, bone alkaline phosphatase; Ca, calcium; P, phosphate; PINP, procollagen type I N-terminal propeptide; NTX, N-terminal telopeptide of collagen; CTX, C-terminal telopeptide of type I collagen; 25OH vitamin D, 25-hydroxyvitamin D serum levels; PTH, parathyroid hormone.

had a rib fracture (mechanism unknown), and the third patient was with lumbar vertebrae L1, pelvis, ribs, and coccyx fractures. All patients, except 1, had a DXA examination: 10 (62.5%) with normal values, 3 had osteopenia (18.8%), and 2 OP (12.5%), of whom 1 had associated multiple skeletal fractures and was receiving antiosteoporotic treatment with denosumab at that time TBS values were also evaluated in most of these subjects, with only 2 patients showing values <1.230 (indicative of degraded microarchitecture) (Table 1).

The parameters of bone metabolism, including bone turnover markers and the genetic study of the 16 patients, are shown in Table 2. Briefly, serum calcium and phosphate levels were within the normal range in all subjects. Five patients (31.3%) presented 25OH-vitamin D levels below 20 ng/mL. It is important to note that bone turnover markers in most patients were within normal range (Table 2), with 1 patient showing slightly elevated values. In addition, most patients (10/15) also showed PINP values over 32 ng/mL (a cut-off value that has recently been proposed to differentiate HPP from low turnover due to antiosteoporotic treatment) [11]. Similarly, the ALP/PINP ratio was under 1.114 in most of the patients evaluated (10/15), being the cut-off value of this ratio also reported to evaluate subjects with HPP [11]. One patient presented elevated parathyroid hormone levels associated with vitamin D deficiency.

Regarding ALP substrates, 5 patients (31.3%) showed elevated PLP levels vitamin B6 >96 nmol/L, none of whom remembered taking vitamin B6 supplementation at that time. A further evaluation of these 5 patients was conducted 36 months later. We again inquired about prior supplementation intake, and several of these patients confirmed having taken supplements at the time of the initial and/or the second analysis. Case 5 recognized the intake supplementation and did not want to repeat the analysis. Case 7 did not remember to have taken any supplements and, at the second study, showed low ALP but normal bone parameters including vitamin B6. Case

8 did not remember to use supplements at the time of the initial assessment but reported current use at the time of second evaluation. Case 11 remembered having taken supplements with vitamin B6, which had been discontinued to perform the second study, showing normalized bone parameters including B6, but still with low ALP. Finally, case 16 reported supplementation with a product with vitamin B6 at first analysis, but the second study was not performed, for unknown reasons.

Genetic analysis revealed no P/LP variants in the *ALPL* gene among the 16 subjects. None of the patients with documented low ALP levels had previously undergone serum ALP substrate measurements, such as vitamin B6, or genetic testing, suggesting that HPP was not suspected.

Discussion

The results of this study showed that in our cohort of patients with FM, 23 (3.2%) presented persistently decreased ALP levels, with 31.25% of the patients studied showed increased PLP substrates (vitamin B6). Although these findings may suggest a HPP, none of these patients had been previously evaluated to rule out this condition prior to the diagnosis of FM.

This aligns with the literature, reporting that the diagnosis of HPP in adults is often delayed by more than 10 years after the onset of symptoms [6, 8, 9, 12, 13]. Indeed, isolated case reports have described patients diagnosed with HPP who were initially misdiagnosed with FM [2, 8]. This may be due to, among other reasons, the fact that the clinical spectrum of HPP is extremely variable, ranging from severe lethal perinatal forms to Odonto HPP and mild adult forms, the latter frequently being overlooked. In addition, the low prevalence of the disease, which ranges from 1/100,000 in North America to 1/300,000 in Europe for severe cases [14], may have played a role in the underdiagnosis. Nevertheless, it should be noted that more recent studies in European descendant HPP patients have estimated a prevalence of mild adult forms as high as 1/508 [14], indicating the need to be aware of this entity, particularly, because physicians not specially dealing with rare bone disease and usually only pay attention to the presence of high and not low serum ALP levels. This is especially important due to the fact that, the adult-onset form of HPP can be highly heterogeneous and may present with several unspecific clinical symptoms like generalized musculoskeletal pain, weakness, fatigue, pseudofractures, or calcific periarthritis, among others [7, 15–18] which can mimic several rheumatologic diseases, such as spondyloarthritis, OP, OA, FM, and other chronic pain syndromes [2, 5–8]. Early recognition and diagnosis of this condition is essential, particularly for correct therapeutic approach in these patients [15]. In this sense, it should be remembered that the use of bisphosphonates should be avoided in HPP since these agents may interfere with mineralization and increase the risk of fractures, such as the atypical femoral fracture, in these patients [3, 15].

Previous studies and isolated case reports have also indicated the need to consider HPP in the differential diagnosis of FM and chronic pain syndromes in adults and include measurement of ALP levels as part of the initial work-up in these patients [2, 7, 8, 14]. In a retrospective study of 611 subjects with FM, persistently low ALP levels were observed in 9.3% of patients, but neither genetic analysis nor ALP substrates were analyzed [7]. Another retrospective study of 9,522 patients from a Rheumatology Outpatient Clinic identified a total of 23 patients with repeatedly low ALP levels. These patients showed musculoskeletal symptoms (symptoms also related to FM, chronic low back pain, tendinitis, OA, spondyloarthropathy, or undifferentiated connective tissue diseases). No secondary causes for decreased ALP measurements were observed, and P/LP variants in the *ALPL* gene were detected in 56.5% of these 23 patients [19]. Finally, a 10-year retrospective study reported persistently low ALP values in 0.32% of hospitalized patients in a Rheumatology and Internal Medicine Department, with 6% having genetically proven HPP [20]. In our series, also retrospectively analyzed, 3.2% of patients presented persistently decreased ALP levels, but the genetic study was negative in all the subjects analyzed. Although the reasons for such discrepancies are unknown, the differences in the population characteristics may have played a role (i.e., our series included only patients diagnosed with FM from a specialized FM Unit, whereas in these latter studies, all subjects from a Rheumatology Department were included).

The fact that diffuse musculoskeletal pain, weakness, and fatigue constitutes the most frequent clinical manifestations of HPP in adults [7, 15–17], further emphasizes the importance of considering HPP in rheumatologic patients with persistently low ALP levels of unknown etiology. As known, HPP is caused by P/LP variants of the *ALPL* gene that encodes for Tissue Non-Specific Alkaline Phosphatase (TNSALP2). This enzyme is expressed in multiple organs including the skeleton, developing teeth, liver and kidney [3]. Reduced ALP

function causes an increase of its substrates, which are three phosphoric compounds: Pyridoxal-5′-phosphate (PLP), phosphoethanolamine (PEA), and inorganic pyrophosphate (PPi). Among these substrates, measurement of serum PLP levels (vitamin B6) is considered a sensitive marker of HPP with increased values generally reflecting disease severity [20]. The diagnostic hallmark suspicion of HPP is the presence of persistently low serum ALP levels with the concomitant exclusion of secondary causes of low ALP levels [21]) and is based on clinical manifestations in combination with low ALP activity, elevated levels of ALP substrates (particularly PLP), and, when possible, genetic confirmation of a causative *ALPL* variant [15, 21]. An international working group on HPP has proposed several diagnostic criteria to improve the diagnosis of HPP in adults with low ALP levels. The authors proposed two major diagnostic criteria for diagnosing HPP: having an *ALPL* gene variant, elevation of natural substrates of TNSALP, recurrent metatarsal stress fractures, and/or atypical femur fracture or having one major and two minor criteria (poorly healing fractures, chronic musculoskeletal pain, early atraumatic loss of teeth, chondrocalcinosis, and nephrocalcinosis) [12]. According to these diagnostic criteria, nearly one-third (31.5%) of our patients with persistently decreased total ALP levels could have been diagnosed with a probable HPP, based on increased *ALPL* substrates and clinical findings. It is important to note that none of the 5 patients who showed increased *ALPL* substrates (vitamin B6), recalled taking dietary supplements, which could be partly justified by the cognitive symptoms accompanying FM. However, at the follow-up control, 3 of these patients acknowledged using a dietary supplement which, after review, contained vitamin B6 in all cases. Therefore, it seems crucial to consistently inquire about the use of dietary supplements in FM patients since they tend to frequently use them [22]. In addition, the composition of the dietary supplements they are taking should also be determined.

Of interest, a recent study has also analyzed the role of bone turnover markers in the diagnosis of adult HPP, particularly if these markers can help differentiate HPP patients from those with OP on antiresorptive treatment [11]. After evaluating markers, such as BAP, PNIP, and CTX, together with the evaluation of total ALP, they also developed several ratios between these bone turnover markers and ROC curve analysis to evaluate the discriminatory power of individual bone markers and their ratios in distinguishing HPP patients from osteoporotic subjects with low ALP activity. These markers showed a satisfactory discriminatory power, particularly with PINP, obtaining an area under the curve of 0.962 with a cut-off of 32 mg/L for this marker and with the total ALP/PINP ratio, the latter with an area under the curve of 0.967 with a cut-off of 1.114, therefore suggesting that bone turnover markers, along with clinical and radiological characteristic, may play a role in the diagnostic workup of HPP [11]. It is noteworthy that in our series, majority of the studied patients (10/15) showed PINP values over 32 ng/mL (the cut-off value proposed to differentiate HPP from low turnover due to antiosteoporotic treatment) as well as a ratio under 1.114 (10/15), both of which would be indicative of HPP. In addition, it has also been suggested that the measurement of BMD in HPP may contribute to discriminating HPP-associated fracture risk from other causes of increased risk like OP since, contrary to OP, BMD is not systematically reduced in patients with fractures [23]. Conversely, increased BMD values appear to be associated with an increased risk for HPP-related fractures in these patients [23]. In our series, most patients showed normal BMD values (10/15 [62.5%]), with 3 (18.8%) and 2 (12.5%) of the subjects analyzed showed osteopenia and OP, respectively, the latter being those with associated skeletal fractures.

However, none of the 16 patients of our cohort showed P/LP variants in the *ALPL* gene study, suggesting that HPP can be overdiagnosed in subjects with FM. Although non-coding *ALPL* variants, mosaicism, or technical limitations of Sanger sequencing or MLPA cannot be totally ruled out as possible causes for a negative genetic study in some of these patients, it seems unlikely because Sanger sequencing and MLPA allowed the detection of 95% of all the ALP mutations [19].

It is well known that several associated clinical conditions, such as thyroid disorders, endogenous or exogenous hypercortisolism, mineral deficiencies, as well as drugs, may be associated with a decrease in ALP levels [12, 21] and should be considered before suspecting a case of HPP, especially if the clinical context is not suggestive [12]. Nonetheless, whether or not concomitant comorbidities and/or treatments could have influenced ALP activity in our patients is not known. In this sense, 5 patients had associated hypothyroidism (although all showed normal thyroid hormone levels), and 5 patients had a concomitant autoimmune disease (but only one was treated with low doses of glucocorticoids [5 mg/day of prednisone]), and the 1 remaining patient was receiving antiosteoporotic treatment with denosumab and presented low ALP levels prior to initiating denosumab treatment. All of this suggests that the diagnosis of mild forms of HPP in adults with FM may be particularly challenging and that, in these subjects, particularly in doubtful cases, closer monitoring and

genetic study seem to be indicative. Although genetic testing may not be necessary for the diagnosis of HPP when clinical manifestations and biochemical tests are indicative of the disease, genetic testing is essential to confirm the diagnosis, to ascertain the inheritance pattern and to perform correct genetic counseling. Moreover, genetic testing is considered the standard of care in the diagnostic workup for HPP [15, 24].

Following the completion of this study and in light of our results, it seems reasonable to include the measurement of ALP levels in patients with chronic pain syndromes, such as FM, as part of the initial work-up. In subjects with persistently low serum ALP levels, after secondary causes for this decrease have been ruled out, a determination of a substrate of ALP, such as PLP, is recommended and always informing the patient about the need to discontinue nutritional supplements and/or multivitamins at least 2 weeks before performing the laboratory analysis. If low ALP levels are confirmed, along with elevated vitamin B6, and the patient presents with a compatible clinical picture – such as chronic widespread pain and associated calcific periarthritis or, chondrocalcinosis, stress fractures (particularly in metatarsals or atypical femoral fracture), dental abnormalities, and/or nephrocalcinosis/nephrolithiasis, among others – genetic testing should be performed taking into account the diagnostic criteria for HPP and the broader criteria for FM, in order to achieve an accurate differential diagnosis.

This study has some limitations such as its retrospective and single-center nature which may limit the generalizability of our findings. Additionally, it is important to keep in mind that the genetic analysis performed in this study may have missed variants in intronic or promotor regions that were not studied or that some other mechanisms may be responsible for the decrease of blood ALP levels. Nonetheless, the strengths of this study include the large number of patients in the cohort analyzed, the access to medical records including treatments, radiological imaging, comorbidities, the additional study of the ALP substrates (PLP) in the individuals with persistently decreased ALP levels, and the genetic tests for specific P/LP variants.

Conclusion

In the present cohort of patients with FM, 3.2% presented persistently decreased ALP levels, but they frequently had comorbidities that could have contributed to this finding. Additionally, one-third of these patients presented increased PLP (vitamin B6) levels, most of them taking it as a dietary complement and none presented *ALPL* P/LP variants, indicating the need to consider this finding in these subjects. Thus, the diagnosis of HPP in subjects with FM should be made with caution and include a complete clinical and laboratory evaluation.

Statement of Ethics

The study was reviewed and approved by the Ethics Committee of the Hospital Clinic of Barcelona, Approval No. HCB/2024/0142. Written informed consent was obtained from participants before entering the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Tamara L. Rodríguez-Araya, Anastasia Mocritcaia, Eva González-Roca, María B. Busso, Chafik A. Chacur, Luciano Polino, Collin D. Adao, Helena Flórez, and Pilar Peris designed the study and analyzed the data. Tamara L. Rodríguez-Araya, Anastasia Mocritcaia, Xavier Torres, Anna Arias, and Pilar Peris analyzed the data and prepared and reviewed the manuscript. All authors approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available on reasonable request from Tamara L. Rodríguez-Araya.

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