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【 CASE REPORT 】

Effect of Asfotase Alfa on Muscle Weakness in a Japanese Adult Patient of Hypophosphatasia with Low ALP Levels

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Abstract:
A 40-year-old Japanese woman presented to our hospital with general fatigue and muscle weakness. She had a history of premature loss of deciduous teeth at 4 years old, her serum alkaline phosphatase (ALP) activity was as low as 91 U/L, and radiologic studies revealed thoracic deformity and sacroiliac calcification. Genetic sequencing revealed a heterozygous c.1559delT mutation in the tissue non-specific alkaline phosphatase gene (*ALPL*). Based on these findings, she was diagnosed with hypophosphatasia (HPP), and treatment with asfotase alfa, a recombinant human tissue-nonspecific alkaline phosphatase (TNSALP), was initiated. After six months of treatment with asfotase alfa, improvements were observed in the SF-36 score, six-minute walk distance, and grasping power. Although the overdiagnosis needs to be avoided, HPP should be considered in patients with undiagnosed musculoskeletal symptoms and a low serum ALP activity.

Key words: hypophosphatasia, bone metabolism, asfotase alfa, alkaline phosphatase

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Introduction

Hypophosphatasia (HPP) is an inherited systemic disorder that affects mineralization in bone and is caused by mutations involving the alkaline phosphatase (*ALPL*) gene, which encodes tissue non-specific alkaline phosphatase (1-3). As of August 2019, *ALPL* is the only reported gene to be associated with HPP, and there are 392 *ALPL* mutations. Although the mode of inheritance varies depending on the mutation, it is inherited as an autosomal recessive disorder in most cases. HPP is frequently caused by the p.E191K and p.D378 V mutations in Caucasian patients, whereas the c.1559delT and p.F327L mutations are more common in Japanese patients (4).

The symptoms of HPP are diverse and depend on the age of onset and type of disease (2, 3). Adult-onset HPP is typically diagnosed based on muscle pain, joint pain, weakness, recurrent stress fractures, and dental abnormalities. Most patients have a childhood history of the premature loss of deciduous teeth or fractures. However, some patients present with less specific symptoms, such as general fatigue and chronic pain, which makes it difficult for clinicians to correctly diagnose these patients (5, 6). In addition, the disease entity has not yet been widely recognized by clinicians. Due to the discrepancy between the absence of reports in Japan and cases already published in other countries, undiagnosed adult cases of HPP may be more frequent than originally considered.

We recently encountered an adult patient with progressive general fatigue and muscle weakness who was diagnosed with HPP based on the findings of low serum ALP activity and radiological bone abnormalities and with HPP who has been successfully treated with enzyme replacement therapy.

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Case Report

A 40-year-old Japanese woman had had general fatigue and muscle weakness since her youth. She had run more slowly than other children since kindergarten, and her walking distance without rest progressively decreased each year. She had visited several hospitals but never been accurately diagnosed. However, the findings of blood examinations in her medical records consistently showed a low serum ALP activity. Based on an internet search, she suspected that her symptoms were compatible with those of HPP.

She had a positive history for the premature loss of deciduous teeth at the age of four but no prior fractures or other illnesses during her development. Her daily diet was well balanced, and she had not taken any regular medications or supplements, thereby ruling out the possibility of a vitamin deficiency. Her height and body weight were 150 cm and 50 kg, respectively, without recent changes. Her elderly brother was alive with no apparent disability, and his serum ALP activity was also low. Her father, who had died of gastric cancer at 66 years old, had also had a low serum ALP activity. In contrast, her mother was healthy with a normal serum ALP activity (Fig. 1).

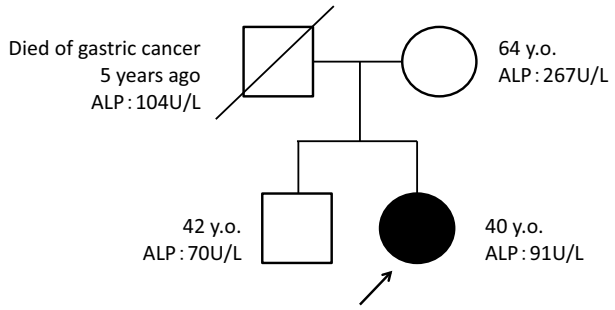

Figure 1. Family tree of the patient. The serum ALP levels of the patient, her father, and elder brother were low, whereas those of her mother were normal.

A physical examination revealed generalized and symmetrical mild muscle weakness (manual muscle test 4/5 in the upper and lower extremities). Her laboratory test results are shown in Table 1. Serum ALP was low at 91 U/L (normal range: 109-200 U/L). Bone-specific ALP was not checked. Total corrected calcium and phosphate as well as intact parathyroid hormone (PTH) levels were 8.5 g/dL, 3.6 mg/dL, and 45 pg/mL, respectively, and were all within normal ranges. Thyroid function tests were normal. The erythrocyte sedimentation rate was also normal. Her urine phosphoethanolamine (PEA) level, which is a supportive marker for the diagnosis of HPP, was within the normal limits.

Computed tomography showed mild thoracic deformity and sacroiliac calcification (Fig. 2). Her lumbar bone mineral density (BMD) was normal (T-score=–0.9 SD), and the serum bone absorption markers tartrate-resistant acid phosphatase (TRACP)-5b and NTX were low, suggesting no apparent bone metabolism abnormalities. Her respiratory function was also normal. Her six-minute walk distance was 137 meters, and her grasping power was 12.6 kgw on the right and 14.6 kgw on the left. Her SF-36 score (Short Form 36 Health Survey), a questionnaire that measures the physically and emotionally based quality of life (QOL) (7), was 10.3 out of 100, with a mean of 50 being the Japanese National standard value (Table 2).

Although these results did not strongly indicate a diagno-

Table 1. Laboratory Examination Findings on Admission.

		Reference range			*Reference range*			*Reference range*
WBC	5,700 /μL	3,000-8,500	TP	7.3 mg/dL	6.7-8.3	Ca(Albumin-corrected)	9.1 g/dL	8.7-10.3
RBC	450 ×10⁴/μL	378-499	Alb	4.6 mg/dL	4.0-5.0	P	3.6 mg/dL	2.5-4.7
Hb	13.8 g/dL	10.8-14.9	AST	18 U/L	13-33	intactPTH	45 pg/mL	10-65
Hct	40.6 %	35.6-45.4	ALT	21 U/L	6-27	1,25-$(OH)_2$VitD	75.2 pg/mL	20.0-60.0
PLT	17.9 ×10⁴/μL	15.0-36.1	ALP	91 U/L	115-359	25-(OH)VitD (After Tx)	9.8 ng/mL	>30
			LDH	176 U/L	119-229	Zinc	85 μg/dL	80-130
			γGTP	28 U/L	10-47			
			ChE	191 U/L	214-466	TSH	1.538 μU/mL	0.34-4.22
CRP	0.05 mg/dL	0.00-0.14	CK	88 U/L	45-163	freeT3	1.21 pg/mL	2.24-3.94
ESR	7 mm/1hr	<16	BUN	16.3 mg/dL	8.0-22.0	freeT4	3.05 ng/dL	0.77-1.59
Ferritin	14.6 ng/mL	12-60	Cre	0.72 mg/dL	0.40-0.70	IGF-1	154 ng/mL	98-245
C3	94 mg/dL	68-128	Na	138 mEq/L	138-146			
C4	19 mg/dL	13-36	K	3.9 mEq/L	3.6-4.9	TRACP-5b	261 mU/dL	120-420
CH50	49 U/mL	32-48	Cl	103 mEq/L	99-109	Urine NTX	41.5 nmolBCE/mmolCr	9.3-54.3
			T-Chol	189 mg/dL	128-219			
						Urine Phosphoethanolamine	57.4 nmol/mgCr	31-110

ALP: alkaline phosphatase, ALPL: alkaline phosphatase gene, BMD: bone mineral density, HPP: hypophosphatasia, MHLW: the Ministry of Health, Labour and Welfare of Japan, PEA: phosphoethanolamine, PLP: pyridoxal 5'-phosphate, PMDA: the Pharmaceuticals and Medical Devices Agency of Japan, PPi: inorganic pyrophosphate, PTH: parathyroid hormone, QOL: quality of life, TNSALP: tissue-nonspecific alkaline phosphatase, TRACP-5b: tartate-resistant acid phosphatase-5b

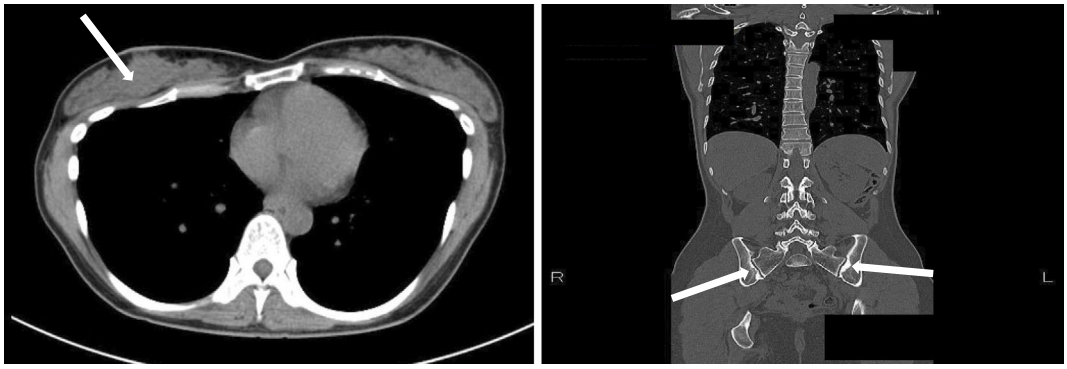

Figure 2. **Computed tomography scans. Left: A deformity of the right chest wall (arrow). Right: bilateral calcification of sacroiliac joints (arrow).**

Table 2. **Change in Clinical Parameters before and after the Administration of Asfotase Alfa. The Muscle Power, Walking Distance, and QOL Score were Improved.**

	Before Tx	*After Tx*
Grasping power (kg)		
Right	12.6	22.1
Left	14.6	17.3
Six-minute walk distance (m)	137	258
SF-36 score		
Total	10.3	47.5
Sub-domain		
Physical Functioning	20	45
Role Physical	0	50
Bodily Pain	22.5	55
General Health Percept	5	45
Vitality	0	31.3
Social Functioning	0	50
Role Emotional	0	50
Mental Health	10	55
T-score in lumbar spine	- 0.9SD	- 1.1SD
Respiratory function test		
%VC(%)	103.8	N.D.
FEV_1%(%)	88.3	N.D.

sis of HPP, her consistently low ALP activity prompted us to suspect HPP. Laboratory and imaging findings lowered the possibility of other diseases, such as osteoporosis, osteomalacia, hyperparathyroidism, and pseudogout. To establish a diagnosis, the whole coding region of the *ALPL* gene was sequenced by the Sanger method using a DNA sample extracted from the blood lymphocytes of the patient. A genetic analysis revealed two heterozygous mutations: c.1559delT and c.258G>A(p.Arg86Arg). Since c.1559delT is the most frequent *ALPL* mutation in the Japanese population and the c.258G>A mutation is a silent mutation, genotyping results suggested that the heterozygous c.1559delT mutation in this patient affected the ALP activity. Based on the premature loss of deciduous teeth, HPP-compatible symptoms, low serum ALP activity, and the genetic mutation in the *ALPL* gene, we diagnosed the patient with HPP.

Treatment with asfotase alfa (Strensiq®) at 0.1 mg/kg subcutaneously per week was initiated. Six-month treatment with asfotase alfa improved not only the subjective symptoms but also the objective findings, including the six-minute walk distance and grasping power (Table 2). The bone density and respiratory function remained largely unchanged. No adverse effects have been reported to date.

Discussion

We encountered a Japanese woman with newly diagnosed adult HPP who was successfully treated with asfotase alfa, a recombinant human TNSALP. HPP has clinically been classified based on the age at presentation into perinatal, infantile, childhood, and adult types. The perinatal and infantile types are generally severe and life-threatening (2, 3, 8), whereas the adult type is less severe.

In their experience of more than 200 pediatric cases of HPP, Whyte et al. reported that the parents carrying one *ALPL* mutation often presented with modest reductions in the serum ALP activity but typically did not show specific signs or symptoms of HPP (9). Therefore, they argued that clinicians should be careful when making the diagnosis of HPP in patients with only a non-specific phenotype (9).

In Japan, the diagnostic criteria of HPP were defined by the Research Committee of Intractable Diseases provided by the Ministry of Health, Labour and Welfare of Japan (MHLW) (10). Since the presence of a low serum ALP activity, the premature loss of deciduous teeth, and the *ALPL* gene mutation in this patient met the MHLW diagnostic criteria of HPP, she was diagnosed with HPP. Furthermore, a thoracic deformity and ectopic calcification, which are relatively specific signs of HPP, were detected, thereby supporting the diagnosis of HPP disease in the present case. The majority of previously reported cases of adult HPP had bone symptoms, such as osteoporosis, fractures, or calcium pyrophosphate deposition. Based on the findings of a patient-reported survey conducted by Weber et al., the main symptoms of adult HPP are muscle weakness (62%), an unusual gait (52%), delayed walking (38%), a short stature (36%), and flexible joints (30%) (11).

A low serum ALP activity is critical for the diagnosis of HPP and is generally used as a screening marker in the search for undiagnosed cases of HPP (12). Clinicians who encounter a patient with a low serum ALP activity and bone symptoms need to consider HPP. A low ALP activity leads to elevated levels of the tissue non-specific alkaline phosphatase (TNSALP) substrates PPi (inorganic pyrophosphate), PLP (pyridoxal 5'-phosphate), and PEA (phosphoethanolamine), which are generally high in HPP patients and used in the next step of the diagnostic procedure (3). Serum PLP is a specific marker for HPP (9) and also indicates the effects of enzyme replacement therapy for HPP, as an elevated PLP means that all TNSALP isoforms are deficient (13). The serum PLP level of the patient after asfotase alfa therapy was 87.8 nmol/L (normal range: 20.5-151 U/L). This result is compatible with that of a patient receiving enzyme replacement therapy (13). In Japan, only urine PEA is commercially available and was within the normal range in our patient.

A definitive diagnosis of HPP is established in the presence of genetic mutations. In the present case, a heterozygous c.1559delT mutation was found in the TNSALP gene. In an *in vitro* study, the deletion of nucleotide 1559 caused a frameshift mutation, which led to the replacement of 3 cysteine residues with serine residues and an additional 80-amino acid extension at the C terminus of the enzyme. The resulting protein product was unable to form the glycosylphosphatidylinositol anchor, so the majority of enzymatic activity was lost (14).

Orimo et al. reported that all Japanese infantile or childhood HPP patients carried c.1559delT as a heterozygote with one more pathogenic mutation at a different locus within the same *ALPL* gene (15). In the present patient, sequencing throughout the coding region of *ALPL*, including introns and exon-intron boundaries, did not result in the identification of any other pathogenetic mutation. Although the c.1559delT carrier frequency has been estimated to be 1/480 in the Japanese population, the heterozygous c.1559delT mutation alone does not appear to be pathogenic in infants and early childhood (16). Based on next-generation sequencing, Taillandier et al. reported that the modifier gene may modulate the phenotype of HPP patients heterozygous for *ALPL* mutations (17). Although we did not perform Sanger sequencing on other genes or a genome-wide analysis, our patient may harbor a mutation in a gene other than TNSALP that is involved in bone mineralization, and this extra mutation may have increased her susceptibility to HPP. Another possibility is that the silent mutation in c.258G>A (p.Arg86Arg) in our patient affected the transcriptional or translational efficiency through epigenetic or RNA interference or other unidentified mechanisms. Further studies to identify unknown mutations or polymorphisms may deepen our understanding of the pathogenesis of HPP.

A treatment has not yet been established for adult cases of HPP. Shapiro et al. recently advocated a treatment for adult patients based on an episode of childhood involvement plus one or more of the following symptoms: 1) musculoskeletal pain, 2) disabling polyarthropathy or chondrocalcinosis, 3) major low trauma fracture, 4) delayed or incomplete fracture healing, 5) repeated episodes of orthopedic surgery, 6) disabling functional impairment, 7) low BMD, and 8) radiological evidence of nephrocalcinosis (18). Since our case satisfied childhood involvement (early milk teeth loss) and disabling functional impairment, we initiated drug therapy.

Asfotase alfa (Strensiq®), a recombinant human TNSALP, was approved for the treatment of HPP by the Pharmaceuticals and Medicals Devices Agency of Japan (PMDA) in 2015. Previous studies reported that asfotase alfa exhibited sufficient and sustained efficacy with safety for perinatal, infantile, and childhood HPP (19, 20). Although studies performed overseas reported the outcomes of HPP treatments using asfotase alfa in adults (21, 22), clinical experience of the treatment of adult HPP cases with asfotase alfa is still insufficient in Japan (19, 23). The present case may serve as a pioneering case and contribute to the wider recognition of the adult disease type of HPP and wider use of therapy in adult cases with long-term symptoms. However, we did not use a placebo, so the data obtained do not exclude the possibility of a placebo effect. Many Japanese adult subjects appear to be heterozygous for this mutation with a slight decrease in serum ALP activity and no HPP disease-associated phenotypes. To prevent the overdiagnosis and overtreatment, the diagnosis of HPP and use of asfotase alpha need to be conducted with special care.

Reported common adverse effects are injection site reactions, hypersensitivity reactions, ectopic calcification, and lipodystrophy (19, 24), none of which were observed in the present case, although longer-term observations will be required.

In conclusion, we encountered the first case of adult HPP in Japan that exhibited marked improvements in symptoms and signs following the initiation of TNSALP enzyme replacement therapy. The present case may serve as an aid to medical professionals for the proper diagnosis and treatment of adult patients with HPP who may otherwise be overlooked.

The authors state that they have no Conflict of Interest (COI).

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