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Case Report

Findings of amplitude-integrated electroencephalogram recordings and serum vitamin B6 metabolites in perinatal lethal hypophosphatasia during enzyme replacement therapy

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Abstract

Hypophosphatasia (HPP) is a rare disorder caused by low serum tissue non-specific alkaline phosphatase (ALP) activity due to hypomorphic mutations in the *ALPL* gene. HPP is characterized by defective bone mineralization. It frequently accompanies pyridoxine-responsive seizures. Because alkaline phosphatase change pyridoxal 5′ phosphate (PLP) into pyridoxal (PL), which can cross the blood brain barrier and regulates inhibitory neurotransmitter gamma-aminobutyric acid. The female patient was born at a gestational age of 37 weeks 2 days. She presented severe respiratory disorder due to extreme thoracic hypoplasia. With the extremely low serum ALP value (14 IU/L), she was clinically diagnosed as HPP. The diagnosis was confirmed with genetic testing. On day1, the subclinical seizures were detected by aEEG. Together with enzyme replacement therapy by asfotase alfa, pyridoxine hydrochloride was administered, then the seizures were rapidly controlled. While confirming that there was no seizure by aEEG monitoring, pyridoxine hydrochloride was gradually discontinued after 1 month. Before administration of pyridoxine hydrochloride, PL was extremely low (4.7 nM) and PLP was increased (1083 nM). After the withdrawal, PL was increased to 84.9 nM only by enzyme replacement. Monitoring with aEEG enabled early intervention for pyridoxine responsive seizures. Confirming increased serum PL concentration is a prudent step in determining when to reduce or discontinue pyridoxine hydrochloride during enzyme replacement therapy.

Keywords: Pyridoxine dependent seizures; Pyridoxal 5′ phosphate; Pyridoxal; Neonatal seizure; Subclinical seizure; Alkaline phosphatase

Abbreviations: HPP, hypophosphatasia; ALP, alkaline phosphatase; PLP, pyridoxal 5′ phosphate; PL, pyridoxal; aEEG, amplitude-integrated electroencephalogram; PEA, phosphoethanolamine.

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1. Introduction

Hypophosphatasia (HPP) is an inborn error of metabolism marked by low serum tissue non-specific alkaline phosphatase (TNSALP) activity due to hypomorphic mutations in the *ALPL* gene [1]. TNSALP is necessary for bone mineralization and vitamin B6 metabolism [1]. Therefore, TNSALP hypoactivity results in skeletal hypoplasia and bone fracture due to hypomineralization of the bone and teeth. Moreover, TNSALP converts pyridoxal 5′ phosphate (PLP) into pyridoxal (PL), which can cross the brood-brain barrier, and regulates inhibitory neurotransmitter gamma-aminobutyric acid (GABA) [2]. Since pyridoxal phosphate acts as a coenzyme for the synthesis of GABA in the brain, the ALP hypoactivity results in a decrease of GABA secretion and of seizure threshold. Hence, functional disorder of TNSALP results in pyridoxine-responsive seizures.

Depending on the age of onset and clinical symptoms, HPP is classified into several subtypes. Perinatal lethal type, the severest form, presents severe respiratory and circulatory failure due to pulmonary hypoplasia and is frequently accompanied by pyridoxine-responsive seizures [3]. Therefore, a suitable method to manage the symptoms is brain monitoring with amplitude-integrated electroencephalogram (aEEG). Recently, enzyme replacement therapy has shown good results in HPP patients [4,5]. In Japan, in August 2015, the therapeutic agent asfotase alfa was approved. There are few reports that describe the vitamin B6 metabolite status and seizure status of the patient before and after asfotase alfa administration. This information is needed to decide when to start and discontinue pyridoxine administration safely.

We report a case of perinatal lethal HPP treated with asfotase alfa and vitamin B6 for pyridoxine-responsive seizures, focusing on the findings of aEEG and the serial measurements of serum vitamin B6 metabolites.

2. Case report

The female patient was born at a gestational age of 37 weeks 2 days to a 32-year-old G3P3 mother. The birth weight was 2614 g. Both the parents were Japanese. There was no family history of consanguineous marriage. The patient's father had a history of bone fracture of the upper arm, clavicle, and pelvis due to traffic accidents. The mother got natural pregnancy and underwent regular prenatal checkup. At the 30th week of pregnancy, the fetal echogram showed shortening of the femurs (−7.0 SD), acoustic shadow attenuation of the bone trunk, and deformation of the cranium upon the application of pressure by the transducer. Since the maternal serum ALP value was 108 IU/L (reference value: 106–322), which is a relatively lower value as compared to the normal values for pregnant women, HPP was suspected. After two prenatal visits, elective caesarean section was scheduled at 37th week of pregnancy to avoid bone fracture and for infant respiratory management. The parents provided informed consent.

At birth, the infant was intubated immediately for the respiratory disorder due to severe thoracic hypoplasia. The body measurements were as follows: height 39.5 cm (−3.2 SD), weight 2614 g (−0.2 SD), and head circumference 37.0 cm (+3.0 SD). The X-ray examination showed a marked decrease of ossification of whole-body bones. No calcification could be identified for the skull except for the skull base (Fig. 1A). Short truncation of the long bone and flaring of the bilateral humerus and femurs were observed (Fig. 1A, B). The ribs were extremely thin (Fig. 1C). The serum ALP value was markedly decreased to 14 IU/L. Serum calcium was within the reference value range of 10.5 mg/dL (reference value: 7.6–11.2 mg/dL). Urinary phosphoethanolamine, which is a substrate of ALP, was as high as 6866.7 μmol/day (reference value: ≤44 μmol/day). For these biochemical examination findings, the patient was clinically diagnosed with perinatal lethal type HPP. Markedly severe respiratory disorder and pulmonary hypertension were managed by intensive care, including administration of fentanyl, and muscle relaxants. Next, enzyme replacement therapy with asfotase alfa was started from day 1.

aEEG monitoring was conducted from day 0. At the beginning of the recording, the aEEG showed a burst suppression pattern with low continuity and the maximal amplitude was 25–50 μV. Electrical seizure clusters were recognized as transient increase of the lower aEEG trace on day 1 (Fig. 2). However, clinical symptoms were not observed. This may be the subclinical seizures or due to sedation and muscle relaxants. Pyridoxine hydrochloride (30 mg/kg) was intravenously injected, after which the electrical seizures quickly disappeared (Fig. 2A). Thus, the seizure was diagnosed as pyridoxine-responsive seizure. Based on the results of aEEG monitoring, pyridoxine hydrochloride administration was gradually discontinued after 1 month and was replaced with enzyme replacement therapy. At the same time, serial measurements of serum vitamin B6, which were PLP and PL, were carried out from birth (Table 1). Serum PLP and PL were measured using high-performance liquid chromatography with fluorescence detection, using precolumn derivatization by semicarbazide [6]. PL was extremely low and PLP was increased before administration of pyridoxine hydrochloride. Serum PLP and PL values were 2414 and 6.9 nM in cord blood and 1083 and 4.7 nM on day 1. However, after pyridoxine hydrochloride and asfotase alfa treatment, both PLP and PL increased to 6401 and 31210 nM, respectively, on day 2. After

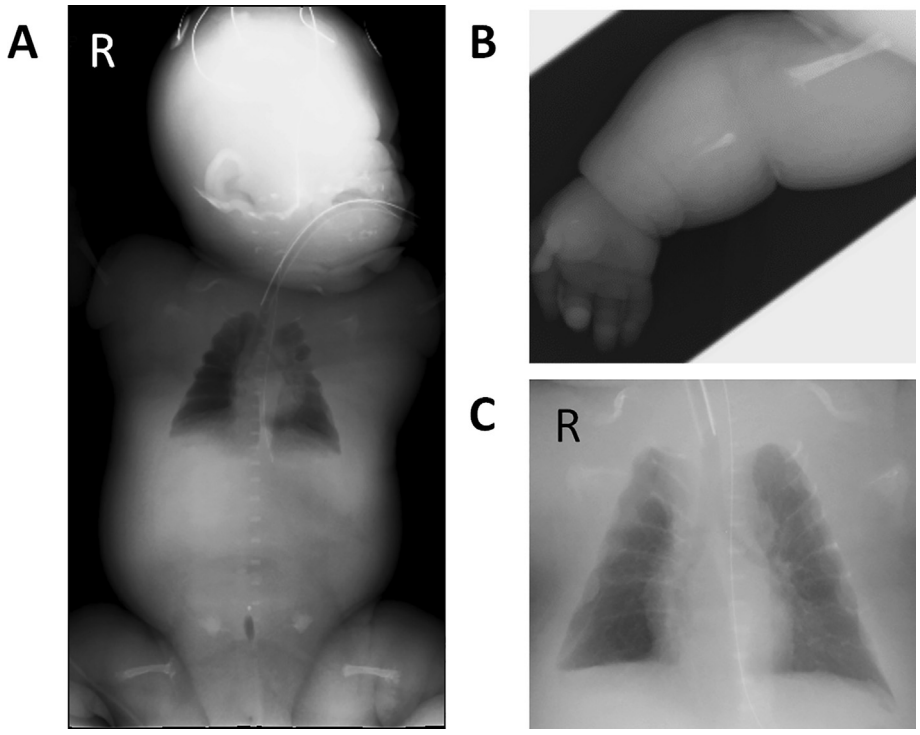

Fig. 1. X-ray photographs at birth. A) Markedly low ossification of the bone and pulmonary hypoplasia were observed. B) Shortening of the right upper limb bone. Flaring of the humerus was observed. C) The ribs were extremely narrow.

discontinuation of pyridoxine hydrochloride, PLP remained at a slightly elevated value and PL was within normal range by the enzyme replacement therapy. PLP and PL values were 92.4 and 84.9 nM on day 33, respectively.

During enzyme replacement therapy, there was no recurrence of seizures. In addition, radiographic findings showed ossification of the whole body, and the shape of the ribs had also improved. However, catheter-related bloodstream infection developed on day 58, and the patient passed away on day 78.

3. Genetic testing

The genetic testing for diagnosis of HPP was approved by the Institutional Review Board of Osaka Women's and Children's Hospital. The parents provided informed consent. By sequencing the *ALPL* gene in this patient, p.H341Q missense mutation of exon 10 was detected in one allele, which is same mutation with her father. This novel mutation is assumed to decrease ALP activity by evolutionary molecular analysis [7]. In the other allele, a frame shift mutation c.1559delT in exon 12 with complete loss of enzymatic activity was found. This mutation is the same with her mother and is the most frequent one in the Japanese population [8].

4. Discussion

Whyte et al. reported that 13 of 37 HPP patients who received asfotase alfa developed pyridoxine responsive seizures [4]. If subclinical seizures are included, there may be more patients who suffered seizures. In our case, aEEG monitoring revealed that electrical seizures can develop within 24 h after birth. The presentation of the seizure was subclinical, but it may be masked by sedative drugs and muscle relaxant. Therefore, it was difficult to recognize without aEEG monitoring. The aEEG enabled early intervention for pyridoxine responsive seizures. As for the statistics in Japan, the developmental prognosis of perinatal and infantile HPP is poor [9]. The early detection and intervention for seizures may improve developmental outcome of HPP patients, especially in this era when curative therapies have developed.

Regarding the effect of enzyme replacement therapy on pyridoxine responsive seizures, Belachew et al. reported that administration of pyridoxine could be discontinued after asfotase alfa administration [10]. In our case, after the increase of serum ALP, pyridoxine could be discontinued without recurrence of seizures. There have been no reports on serial changes of vitamin B6 metabolites during enzyme replacement therapy in HPP. Prior to the administration of pyridoxine and

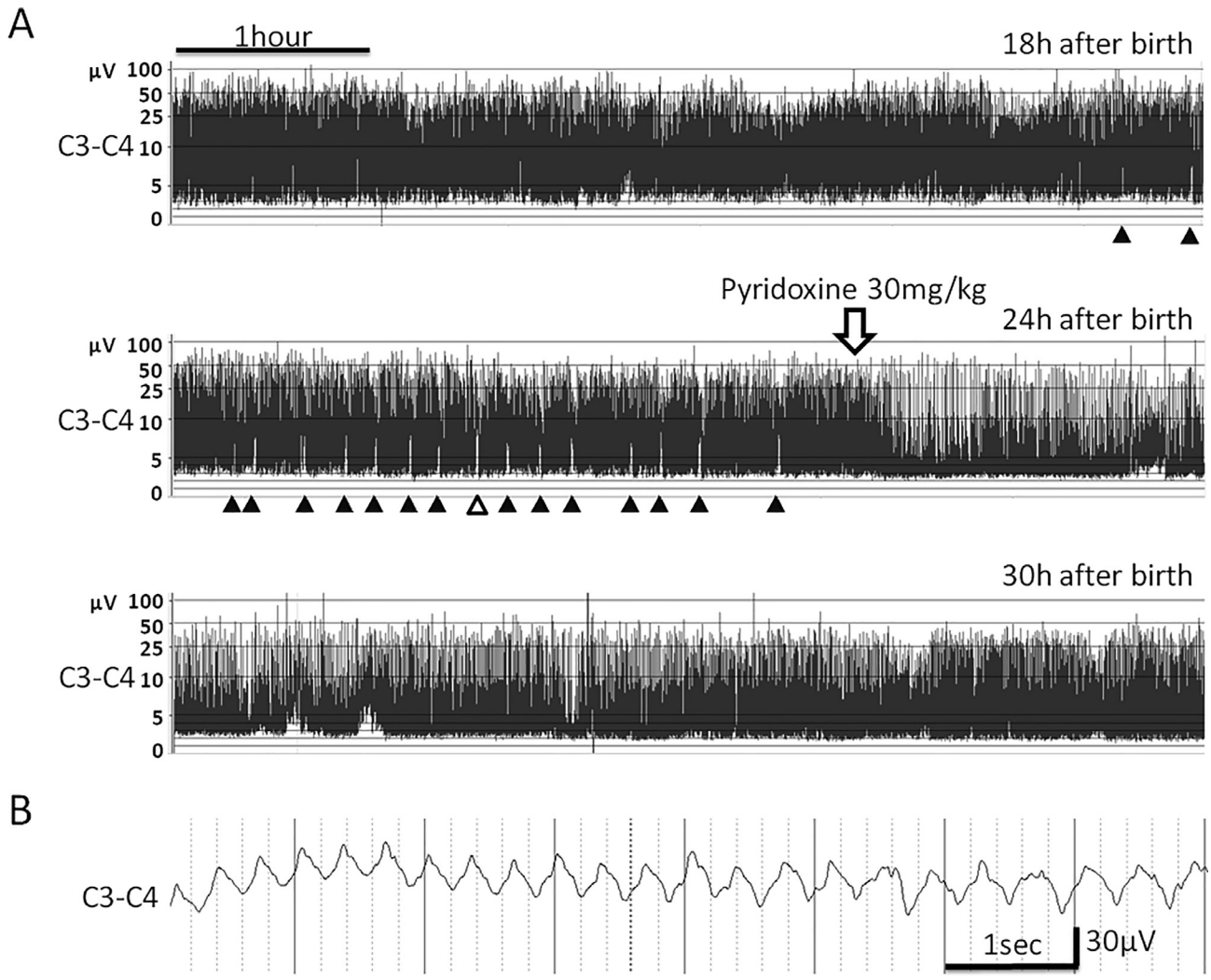

Fig. 2. A) Recording of aEEG from 12 to 30 h after birth. At 12 h after birth, the aEEG background pattern was Burst-Suppression. Seizure patterns were seen from 18 h after birth with transient increase lower margin of the aEEG trace (black and white arrow head). The white arrow head indicates the raw EEG shown in Fig. 2B. The hollow arrow shows the point of pyridoxine hydrochloride administration. Thereafter, the electrical seizures completely stopped. B) Representative raw waveform of the seizure. The electrical seizure showed by rhythmic, repetitive and evolutional waveform.

Table 1
Serial measurements of vitamin B6 metabolite status.

	Cord blood	Day 1	Day 2	Day 25	Day 33
		Before pyridoxine and asfotase alfa administration	2nd day after pyridoxine and asfotase alfa administration	25th day after pyridoxine and asfotase alfa administration	4th day after discontinuing pyridoxine
ALP (IU/L) (reference:106–322)	14	4	42	17,652	11,399
PLP (nM) (reference:21–188)	2414	1083	6401	147.3	92.4
PL (nM) (reference:18–106)	6.9	4.7	31,210	172.7	84.9

ALP; alkaline phosphatase, PLP; pyridoxal 5′ phosphate, PL; pyridoxal.
Reference values are from Akiyama et al. [6].

asfotase alfa, PL was markedly low and PLP was extremely high, which was compatible with severely deficient ALP activity. Both serum PLP and PL values showed a quick and marked increase during pyridoxine and asfotase alfa administration. After discontinuation of pyridoxine, serum PLP was maintained at a mildly high

value and PL was within the normal range. Since asfotase alfa dephosphorylates PLP and increases PL that have crossed the blood-brain barrier, there will be no need of vitamin B6 administration for treatment of seizure. Thus, confirming increased serum PL concentration would be a good reference to determine when to reduce or discontinue pyridoxine hydrochloride during enzyme replacement therapy. In this case, we could discontinue pyridoxine in one month with the help of aEEG monitoring and vitamin B6 metabolite measurement as indicators.

In this case, the patient's respiratory state was so severe that she never got off from the mechanical ventilation. Therefore, it was difficult to take head MRI or to follow neurological development.

5. Conclusion

This case showed that aEEG monitoring was useful for early treatment intervention and safe pyridoxine withdrawal. Moreover, it also showed that perinatal HPP patients can present pyridoxine-responsive seizure even 24 h after birth. Enzyme replacement therapy changed the vitamin B6 metabolism and made it possible to discontinue pyridoxine in one month.

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