# Hypophosphatasia : The Importance of Alkaline Phosphatase in Bone Mineralization

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**Abstract**

The authors describe a neonate who was diagnosed with "perinatal hypophosphatasia". The clinical manifestations in this patient were small head size, soft calvarium (caput membranaceum), and short bowing forearms and legs. Laboratory investigations revealed hypercalcemia at 12.7 mg/dl, hyperphosphatemia 8.6 mg/dl, and extremely low alkaline phosphatase 0 unit/L. Roentgenographic studies of the skull showed calcification only at frontal bone and base of the skull. Spines were small and flattened. Long bones were hypomineralized and deformed. The functions of alkaline phosphatase to bone development and mineralization were reviewed. Because perinatal hypophosphatasia is a fatal condition and inherited as an autosomal recessive pattern, prenatal diagnosis is necessary. The most reliable and suitable method in our facility is serial ultrasonography from which the diagnosis can be made by the second trimester.

**Key word** : Alkaline Phosphatase, Bone Mineralization, Hypercalcemia, Hypophosphatasia

Hypophosphatasia is a rare inherited metabolic bone disease characterized clinically by defective bone mineralization and biochemically by deficient tissue non-specific alkaline phosphatase activity[1,2]. There are six clinical types of hypophosphatasia which vary in severity : perinatal, infantile, childhood, adult, odontohypophosphatasia, and pseudohypophosphatasia[3,4].

The most severe form of hypophosphatasia is the perinatal type which is expressed *in utero* with profound skeletal hypomineralization[1,4]. This disorder can cause intrauterine death, stillbirth, or neonatal death. It is inherited as an autosomal recessive trait. The prevalence is approximately 1/100,000 livebirths[5].

We report here a newborn with perinatal hypophosphatasia. The importance of alkaline phosphatase in skeletal mineralization is reviewed.

## CASE REPORT

The patient was a Thai male neonate born to a $G_2$ $P_0$ $A_1$ 28-year-old mother and 30-year-old

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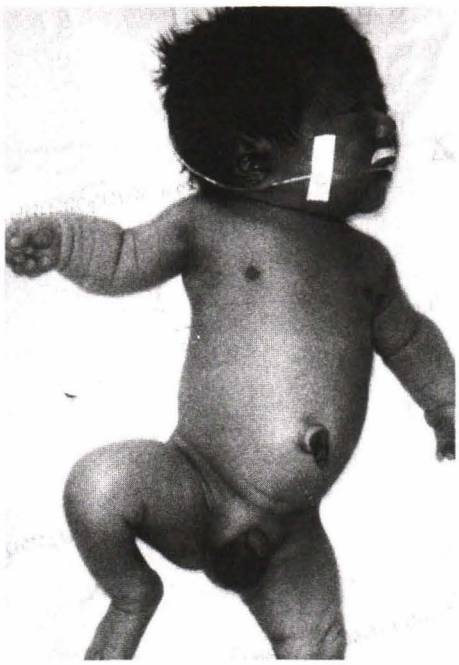

**Fig. 1. Patient phenotype. Facial appearance was normal. Note short deformed forearms.**

father who were unrelated. The first pregnancy, 3 years ago, ended with criminal abortion. The mother had no history of alcohol, cigarette or drug exposure. The patient was born at term by Caesarean section due to breech presentation. Birth weight was 3,250 g. Apgar scores were 9 and 10 at 1 and 5 minutes, respectively. The patient was noted to have short and bowing limbs. Birth length was 45 cm and head circumference was 33 cm. Physical examination revealed normal facial appearance. The calvarium was soft with only the frontal bone palpable. Chest wall was normal. The forearms and lower legs were short with anterior bowing (Fig. 1). The rest of the physical examination was unremarkable.

Laboratory investigation revealed normal electrolytes, but serum calcium and phosphorus were elevated at 12.9 mg/dl and 8.6 mg/dl, respectively. Roentgenographic study of skull bones showed that mineralization was present only at frontal bones and base of the skull (Fig. 2). The spines were small and flattened. Generalized hypomineralization was seen in the long bones. At 7 days of age, serum calcium was still elevated at 12.0 mg/dl, phosphorus 7.5 mg/dl and alkaline phosphatase 0 unit/L. BUN was 8.9 mg/dl, creatinine 0.42 mg/dl, and albumin 4.5 g/dl. Serum levels of alkaline phosphatase done at days 8 and 9 were still at 0 and 0 unit/L. The diagnosis of the perinatal form of hypophosphatasia was made on the basis of clinical, laboratory, and roentgenographic findings. The patient died at 12 days of age from respiratory failure.

## DISCUSSION

The clinical manifestation of skeletal hypomineralization in our patient was present at birth which suggested that the defect of skeletal mineralization started *in utero*. The differential diagnosis for intrauterine hypomineralization were achondrogenesis type I, fibrochondrogenesis, osteogenesis imperfecta type II, and perinatal hypophosphatasia[1,2]. The patients with three former syndromes usually have characteristic facial dysmorphism such as flat facies, low nasal bridge, micrognathia, and extremely small stature associated with severe bone deformities. Large cranium is found in patients with achondrogenesis and fibrochondrogenesis. In osteogenesis imperfecta type II, the patients usually have dark or blue sclerae and a beaked nose. The primary etiology causing these three syndromes is

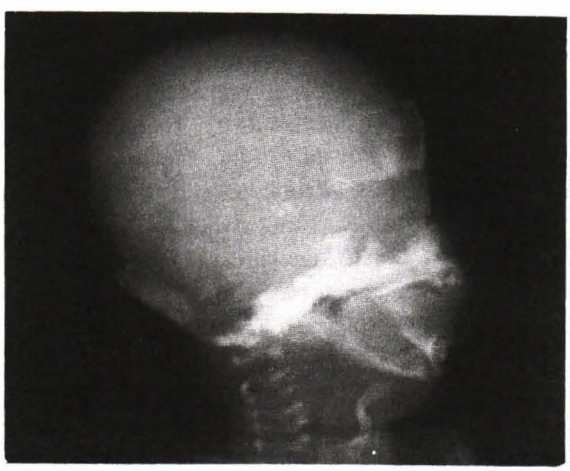

**Fig. 2. Roentgenographic finding of skull. Note the presence of mineralization only at frontal bone and base of skull.**

a defect in collagen synthesis and function, resulting in secondary impairment of skeletal mineralization. In our patient, facial appearance was normal, the head was relatively small, and limb deformity was not extremely severe. The absence or extremely low level of serum alkaline phosphatase associated with hypercalcemia in our patient is an important clue that led to the diagnosis of perinatal form hypophosphatasia.

The clinical evidence of skeletal hypomineralization in patients with hypophosphatasia indicates that alkaline phosphatase is important for skeletal mineralization[1,4]. In man, there are 4 alkaline phosphatase (ALP) isoenzymes encoded by 4 distinct gene loci[6,7]. Three of the ALP isoenzymes are each expressed in a tissue specific distribution and are named according to its specific tissue as intestinal, placental, and germ cell ALP. The fourth ALP isoenzyme is ubiquitous, but especially abundant in hepatic, skeletal, and renal tissue. Thus, the liver/bone/kidney ALP is also called tissue non-specific ALP (TNSALP). In normal adults, most of the TNSALP in serum reflects about an equal amount of TNSALP from liver and bone[8]. In growing infants and children, serum is rich in the bone form of TNSALP resulting in much higher levels of ALP than in adults.

The biological functions of TNSALP are numerous such as hydrolysis of phosphate ester, transferase action of steady state levels of phosphoryl metabolites, and regulation of inorganic phosphorus metabolism[6,7]. The three main substrates for TNSALP function are pyridoxal-5-phosphate (PLP), inorganic pyrophosphate (PPi), and phosphoethanolamine (PEA)[3,4]. Therefore, deficiency of TNSALP results in the elevation of these three substrates. The accumulation of PPi, a potent inhibitor of hydroxyapatite crystal growth, is an explanation for defective mineralization of bone and cartilage in patients with hypophosphatasia.

Hypophosphatasia has been classified into 6 forms[3,4]. The age at which lesions are manifested in the bone can distinguish the perinatal, infantile, childhood, and adult types from each other. Odontohypophosphatasia is classified in patients who have only dental manifestation without bony involvement[9]. The term "pseudohypophosphatasia" describes the hypophosphatasia variant in patients who have clinical, radiological, and biochemical resembling infantile hypophosphatasia, but serum ALP is in the normal range[10,11]. The prognosis for these six forms of hypophosphatasia depends upon the severity of the skeletal disease which reflects the age at presentation. The earlier the skeletal symptoms manifest, the more severe the disease[3,4]. The most severe hypophosphatasia is the perinatal form in which the disease occurs in utero and the infant is usually stillborn, or expires a few days after birth from respiratory failure. Profound skeletal hypomineralization results in "caput membranaceum" (membrane-like calvarium) and short deformed limbs[3].

The diagnosis of perinatal hypophosphatasia can be made confidently when the patient has consistent clinical findings, radiological changes and low level of serum ALP[3]. The elevated levels of 3 substrates for TNSALP (PLP, PPi, PEA) support the diagnosis of hypophosphatasia, but are not pathognomonic since the elevation of these substrates can occur in a variety of metabolic bone diseases[12]. Currently, the molecular study of hypophosphatasia can be used for diagnosis. The gene for TNSALP is known to be located near the tip of chromosome 1 (1p36.1-p34)[13]. The gene comprises more than 50 kb of DNA and contains 12 exons[14]. More than 10 points of missense mutations have been reported[15].

Perinatal hypophosphatasia is a fatal disease. Survival may be extended by intensive life support. Enzyme replacement therapy by intravenous infusion of normal plasma, or the plasma of patients with Paget disease did not lead to clinical improvement[16,17]. These observations suggest that the local tissue levels of ALP are greater than in circulation or that ALP must be present locally in the skeleton to be physiologically active for mineralization[18]. This hypothesis is supported by the hypomineralization of perinatal hypophosphatasia in which there is abundant placental ALP in the mother and amniotic fluid, but it can not protect the fetus from the disease.

Hypophosphatasia is an autosomal recessive inherited disease. Hence, prenatal diagnosis is recommended. Serial ultrasonography with careful attention to the skulls and limbs is the most reliable method, but the abnormalities are clearly evident in the second trimester[3,4]. Recently, Warren et al reported a case in which the diagnosis was

made in the first trimester by using a specific monoclonal antibody for TNSALP that would not cross react with placental ALP on a chorionic villous specimen[19]. However, the specific monoclonal antibody is not usually done in Thailand due to the scarcity of diseases. Hence, prenatal diagnosis by serial ultrasonography is recommended at the present time.

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# ฮัยโปฟอสฟาเทเซีย : ความสำคัญของเอ็นซัยม์ อัลคาไลน์ ฟอสฟาเทส ต่อการสร้างกระดูก

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รายงานผู้ป่วยทารกแรกเกิด 1 รายที่ได้รับการวินิจฉัยว่าเป็น perinatal hypophosphatasia ความผิดปกติที่พบในผู้ป่วยรายนี้ ได้แก่ ศีรษะขนาดเล็ก กะโหลกศีรษะบางมากจนสามารถคลำเนื้อสมองได้ (caput membranaceum) กระดูกแขนขาสั้นและโค้งงอ (short bowing forearms and legs) ผลการตรวจทางห้องปฏิบัติการพบว่าระดับแคลเซียมในเลือดสูง 12.9 มก./ดล. ฟอสฟอรัส 8.6 มก./ดล. และ alkaline phosphatase 0 ยูนิต/ล. ภาพถ่ายรังสีบริเวณศีรษะพบว่ามีกระดูกเฉพาะ frontal bone และส่วนฐานของกะโหลกศีรษะเท่านั้น กระดูกสันหลังขนาดเล็กและแบน กระดูกแขนขามีลักษณะบางและผิดรูปร่าง ได้ทบทวนวารสารถึงความสำคัญและบทบาทของเอนซัยม์ alkaline phosphatase ต่อพัฒนาการของการสร้างกระดูกตั้งแต่ทารกอยู่ในครรภ์มารดา ผู้ป่วย perinatal hypophosphatasia ทุกรายเสียชีวิตในวัยทารกแรกเกิด แบบแผนการถ่ายทอดทางพันธุกรรมเป็นลักษณะ autosomal recessive ฉะนั้น การวินิจฉัยก่อนคลอดจึงเป็นสิ่งจำเป็น วิธีที่เชื่อถือได้และเหมาะสมตามสภาพการณ์ของประเทศไทย คือ การตรวจทารกในครรภ์ด้วยคลื่นความถี่สูงเป็นระยะ ซึ่งสามารถวินิจฉัยความผิดปกตินี้ได้ในระยะไตรมาสที่สอง.

**คำสำคัญ** : การสร้างกระดูก, กระดูกบาง, แคลเซียมในเลือดสูง, ฮัยโปฟอสฟาเทเซีย

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