Two Case Reports of FGF23-Induced Hypophosphatemia in Childhood Biliary Atresia

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abstract

Cholestatic liver disease has long been associated with childhood rickets, secondary to impaired absorption of fat-soluble vitamin D. Elevated serum levels of fibroblast growth factor 23 (FGF23), secondary to genetic defects or tumor-induced osteomalacia, causes hypophosphatemic rickets in childhood. We present 2 infants with end-stage liver disease due to biliary atresia (BA) who developed hypophosphatemia with renal phosphate wasting. Serum FGF23 levels were elevated more than 8 times the upper limit of normal, and the older infant showed radiographic evidence of rickets. Both infants required large supplements of phosphate in addition to calcitriol. Following liver transplantation, FGF23 normalized in both patients and phosphate and calcitriol supplementation were discontinued. Immunohistochemistry revealed ectopic overexpression of FGF23 by hepatocytes in the BA liver. These observations highlight a unique cause of hypophosphatemic rickets in childhood and suggest the need for further investigation into the relationship between BA and other cholestatic disorders, and bone metabolism.

In pediatric patients with cholestatic liver disease, decreased concentrations of bile acids in the small bowel lumen result in intestinal malabsorption of fat-soluble vitamins that increases the risk for vitamin D–deficiency rickets.[1,2] Severe vitamin D deficiency may result in hypophosphatemia in addition to hypocalcemia; however, renal reabsorption of phosphorus is maximized. Elevated fibroblast growth factor 23 (FGF23), a phosphaturic factor, is the cause for hereditary hypophosphatemic rickets (HHPR) and, less commonly, tumor-induced osteomalacia.[3–6] We report 2 patients with severe cholestasis and biliary cirrhosis who developed hypophosphatemia and urinary phosphate wasting, with the older patient also displaying skeletal manifestations of rickets. These cases are unique, as they demonstrate increased FGF23 as a cause of hypophosphatemia in pediatric cholestatic liver disease.

CASE 1

An ill-appearing African American girl presented to an outside institution at 9 months of age with jaundice, tachycardia, protuberant abdomen with ascites, hepatosplenomegaly (spleen and liver 7–8 cm below costal margin), caput medusae, and diffuse muscle wasting. She was born at term without neonatal complications, was breastfed with formula supplementation, but did not receive vitamin supplementation. Poor growth was noted between 6 and 9 months of age. The family history

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TABLE 1 Anthropometric Parameters and Laboratory Values at Presentation With Hypophosphatemia

	Reference Range	Case 1	Case 2
Age, mo	—	9	6
Weight, percentile	—	32nd	2nd
Length, percentile	—	11th	<1st
Head circumference, percentile	—	19th	<1st
Triceps skin fold thickness	—	4 mm (<5 percentile)	5 mm (<5 percentile)
Laboratory values: bone metabolism			
Albumin corrected calcium, mg/dL	8.0–10.5	9.3	8.7
Serum phosphorus, mg/dL	2.8–6.9	1.5	1.9
Serum magnesium, mg/dL	1.7–2.4	2.0	2.2
Alkaline phosphatase, U/L	110–345	714	379
Parathyroid hormone, pg/mL	6–89	94	16
25 OH-D, ng/mL	20–60	12	13
1,25 OH-D, mg/dL	27–71	18	55
TRP, %	>90	38	33
TmP/GFR, mg/dL	3.6–5.4	0.57	0.63
Laboratory values: liver function			
Total bilirubin, mg/dL	0.1–1.1	8.9	9.4
Direct bilirubin, mg/dL	0.0–0.3	6.9	5.2
γ-glutamyl transferase, U/L	2–19	134	1201
Alanine aminotransferase, U/L	12–49	61	173
Aspartate aminotransferase, U/L	16–62	118	366
INR	—	1.43	1.1
PELD	10–40	11	25

INR, international normalized ratio; PELD, Pediatric End-Stage Liver Disease Score; TmP/GFR, tubular maximum phosphate reabsorption per glomerular filtration rate, calculated as TRP × phosphorus when TRP ≤86%.

was negative for hypophosphatemia, bowing, fractures, or metabolic bone disease.

Liver biopsy performed at the referring institution showed complete bridging fibrosis, ductular proliferation, and bile plugs consistent with large bile duct obstruction. Magnetic resonance imaging showed large intrahepatic cystic structures in the hilum, possibly involving the extrahepatic bile ducts. The patient was transferred to our hospital for liver transplant evaluation.

Table 1 presents anthropometric parameters, serum, and urinary laboratory values obtained at our institution. Because of the hepatic cystic changes and renal involvement with phosphate wasting,[7,8] the possibility of Caroli disease (ectasia/segmental dilatation of large intrahepatic ducts) was considered. Renal ultrasound showed bilateral kidney enlargement without cystic changes. Parenteral nutrition was initiated with resultant decline in serum phosphorus, suggesting refeeding syndrome, although electrolyte abnormalities did not improve with decreased caloric intake. Phosphorus replacements up to 140 mg/kg per day in 4 divided doses and ergocalciferol (8000 IU daily) were required to maintain normal serum phosphorus levels. Radiographs showed evidence of rachitic changes with periosteal reaction and a healing fracture of the left distal femur (Fig 1). Blood obtained 4.5 hours after a phosphate bolus showed marked elevation of plasma c-terminal FGF23 concentration at 1910 RU/mL (reference range <230 RU/mL), whereas phosphorus was 2.1 mg/dL (reference range 2.8–6.9 mg/dL). Calcitriol (47 ng/kg per day) was added, and all supplements were continued until the patient underwent orthotopic liver transplantation at 13 months of age.

Examination of the explanted liver showed biliary cirrhosis with duct proliferation and bile plugs,

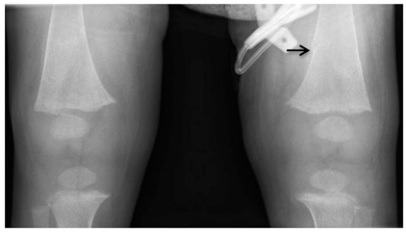

FIGURE 1
Bilateral knee radiograph of case 1. Radiographs show thin cortices and subperiosteal resorption, mild metaphyseal fraying of the femur and tibia bilaterally with a relative lack of provisional calcification, and a nondisplaced, healing fracture of the left distal femur (arrow).

but no evidence of ductal plate malformation, no large malformed bile ducts (as seen in Caroli disease), or tumor. Many large pseudocysts (bilomas) were concentrated in the hilar region of the liver, where a bile duct remnant consisting of a dense fibrous cord was identified within the hilar fibrous plate. Based on these findings, a diagnosis of extrahepatic BA was established. Three weeks posttransplant, FGF23 was normal (223 RU/mL) and tubular resorption of phosphate (TRP) normalized to 97%. Phosphorus supplementation was gradually decreased and serum phosphorus stabilized in the normal range. Ergocalciferol and calcitriol were also discontinued. Follow up radiographs showed periosteal new bone formation and healing rickets.

CASE 2

A white boy with a history of extrahepatic BA (Kasai hepatoportoenterostomy at 2 months of age) presented at 6 months of age with persistent jaundice, progressive hepatosplenomegaly, portal hypertension, failure to thrive, and significant hypophosphatemia and renal phosphate wasting (Table 1). Plasma c-terminal FGF23 was increased at 7915 RU/mL (reference range <230 RU/mL). Intravenous phosphate boluses were given, and the patient was further treated with 20 mg/kg of enteral phosphate in

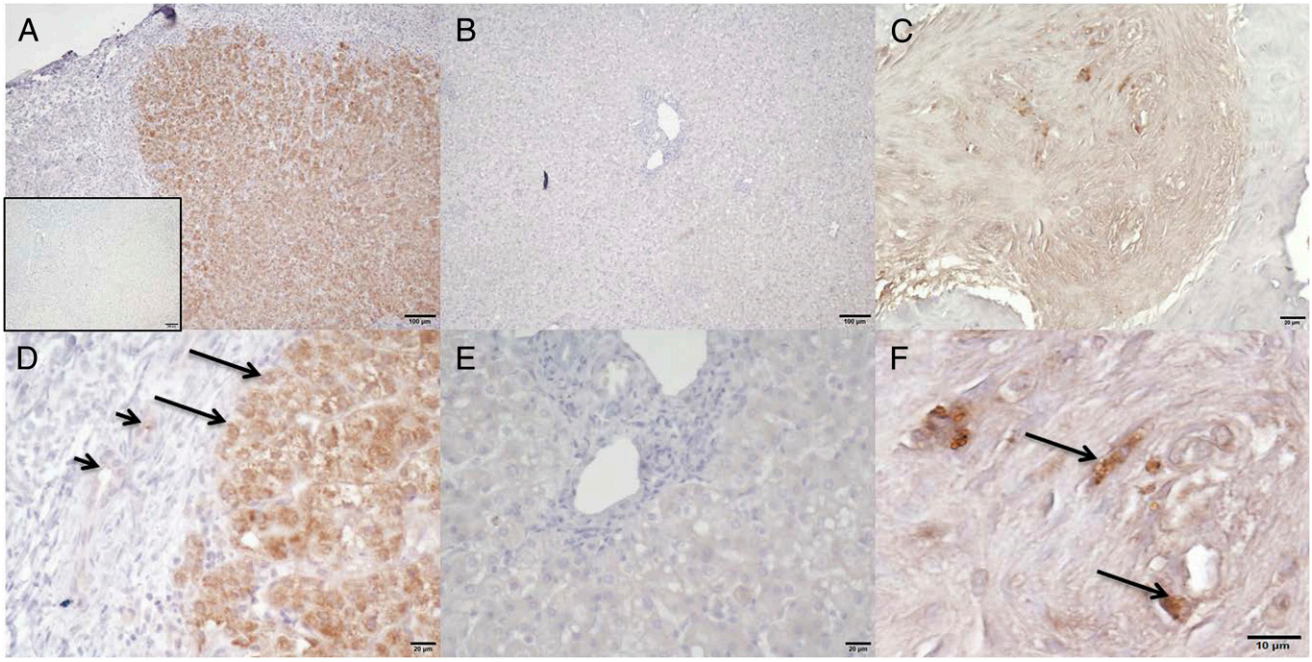

FIGURE 2

Expression of FGF23 in liver tissue from case 2 obtained at the time of Kasai portoenterostomy. Hepatocytes are immunoreactive for FGF23 throughout the lobule (A). Omitting of the aFGF23 in a control experiment produces negative results (A; insert). Higher magnification reveals granular pattern of cytoplasmic staining in hepatocytes (D; arrows) and faint staining in the bile duct epithelium (D; arrowhead). Liver tissue from the background liver of an infant undergoing resection of benign liver lesion serving as control is negative for significant staining in lower (B) and higher magnification (E). Biopsy material from osteoma of patient with MAS serves as biological positive control (C). At higher magnification (F), arrows denote FGF23 positive osteoblasts.

4 divided doses plus phosphate supplementation of 35 mg/kg per day in total parental nutrition. He had been receiving supplementation with 8000 IU ergocalciferol daily for significant hypovitaminosis D. Skeletal radiographs showed no evidence of rickets or secondary hyperparathyroidism.

Eleven days after orthotopic liver transplantation, plasma FGF23 had normalized to 84 RU/mL. Enteral feeds were resumed, ergocalciferol supplementation was reduced to 4000 IU once daily, and phosphorus supplementation was discontinued while serum phosphorus stabilized in the low normal range for age (3.7–4.2 mg/dL).

IMMUNOHISTOCHEMISTRY: MATERIALS AND METHODS

Research studies on archived liver tissue were performed under a protocol approved by the institutional review board at our institution. Paraffin-embedded liver tissue was deparaffinized and rehydrated; antigen retrieval was performed with pressure cooker in sodium citrate pH 6.0. Primary antibody, goat antihuman FGF23 (Santa Cruz Biotechnology, Santa Cruz, California; SC27249; 1:20 vol/vol dilution) was applied overnight at 4°C, and secondary biotin-conjugated antibody donkey antigoat (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania; 1:5000 vol/vol dilution) was applied for 4 hours at room temperature. The primary antibodies were omitted for negative control. Decalcified archived bone tissue from patients with McCune-Albright syndrome (MAS) and fibrous dysplasia were used as positive controls.

IMMUNOHISTOCHEMISTRY: RESULTS

Under physiologic conditions, FGF23 is expressed by osteoblasts and osteocytes. Overexpression of FGF23 has been detected by immunohistochemistry in osteoblasts within fibrous dysplastic bone of patients with MAS and renal phosphate wasting.[9] To identify the cellular source for circulating FGF23 in our patients, we performed immunohistochemical studies on sections from paraffin-embedded liver tissue obtained from case 2 at the time of Kasai portoenterostomy. In the diseased liver, moderate anti-FGF23 immunoreactivity in granular pattern was observed in the cytoplasm of hepatocytes in a panlobular distribution (Figs 2A and 2D). Faint focal staining was observed in cholangiocytes (Fig 2D). FGF23 expression was near absent in anti-FGF23–stained sections from normal liver tissue of infants without cholestatic disease (Fig 2B/E). Immunohistochemistry of archived fibrous dysplasia tissue from a patient with MAS revealed strong immunoreactivity in fibrous bone cells (Fig 2C/F).

DISCUSSION

This is the first report of hypophosphatemia associated with increased FGF23 production in pediatric patients with end-stage liver disease (ESLD) due to BA and progressive cholestasis. As previously described in MAS, serum FGF23 corresponded with serum phosphate concentrations,[10] and hypophosphatemia improved with normalization of FGF23 after liver transplantation in both cases. In the setting of extrahepatic biliary obstruction, we observed overexpression of FGF23 in hepatocytes.

FGF23 is a key regulator of phosphate homeostasis.[11] In the kidney, FGF23 binds with an essential cofactor, klotho, to inhibit 1-α hydroxylase, stimulate 24-α hydroxylase, and downregulate sodium phosphate cotransporters 2a and 2c at the proximal renal collecting tubules. The result is increased urinary phosphorus excretion and decreased 1,25-dihydroxyvitamin D production.[12,13] Under physiologic conditions, FGF23 expression is enhanced by hyperphosphatemia and high 1,25-dihydroxyvitamin D concentrations.[14,15]

In childhood, hypophosphatemic rickets secondary to increased serum FGF23 is best described in HHPR caused by phosphate-regulating endopeptidase homolog, X-Linked (*PHEX*) gene mutations. Without a known family history of HHPR, patients do not present until early childhood after weight bearing has commenced when progressive lower extremity bowing occurs.[3,16] Radiographic evidence of rickets is typically more pronounced in the lower extremities as opposed to the upper extremities. Other forms of HHPR are due to impaired catabolism of FGF23 or increased FGF23 production through defects in osteocyte maturation and may lead to a similar skeletal phenotype.[17] Rarely, tumor-induced osteomalacia has been associated with hypophosphatemic rickets in children due to increased production of FGF23.[4,5] In all these conditions, chronic hyperphosphaturia, despite low serum phosphate, with normal or low 1,25-dihydroxyvitamin D due to impaired 1-α hydroxylase activity occurs, necessitating phosphorus and calcitriol supplementation to mitigate the effects of the increased FGF23. Our patients had a similar biochemical phenotype, with case 1 also manifesting radiographic evidence of hypophosphatemic rickets; in case 2, we did not observe the skeletal sequelae. The coexistence of vitamin D deficiency may have contributed to hypophosphatemia in both patients and the delayed diagnosis of BA without initiation of fat-soluble vitamin replacement may have also contributed to the skeletal findings in case 1. Given the ages of both patients at diagnosis, it is not surprising that significant rachitic changes were absent despite the observed severe biochemical abnormalities.

Hypophosphatemia secondary to renal tubular defects or isolated vitamin D deficiency is not associated with FGF23 elevation.[18] Although secondary hyperparathyroidism from vitamin D deficiency may cause renal phosphate wasting, it is unlikely that mild elevation of parathyroid hormone seen in case 1 contributed significantly to the severe hypophosphatemia.[19] Additionally, parathyroid hormone and low phosphorus are potent stimuli of 1-α hydroxylase, and patients with hypophosphatemic rickets due to causes other than elevated FGF23 are expected to have high concentrations of 1,25-OH vitamin D.[20]

In a study of adult patients with ESLD awaiting liver transplantation, FGF23 was elevated in 63% of patients (median: 241 RU/mL, range 5–17 620).[21] Univariate analysis indicated FGF23 was a stronger predictor of mortality than classic liver disease severity scoring systems (Model for End-Stage Liver Disease and Model for End-Stage Liver Disease-Sodium). After multivariant analysis, including other known prognostic factors determining survival, FGF23 remained significantly associated with mortality with a hazard ratio of 2.21 (confidence interval 1.67–2.93). FGF23 was interpreted as being biologically active in these patients given that median 1,25-OH vitamin D was at the low end of normal in the vast majority of patients. Biopsy data were not available from these patients. The same authors found increased hepatic FGF23 mRNA in mice with induced liver injury as compared with controls.[21] These studies support the hypothesis that chronic liver disease can lead to increased production of FGF23.

Hypophosphatemia has been reported after hepatic resection in adult patients with elevated c-terminal FGF23 and normal intact FGF23, although neither measure was correlated with urinary phosphate wasting severity.[22] Although FGF23 did not seem to be the causative factor of hypophosphatemia in these patients, c-terminal FGF23 levels were less elevated as compared with our cases. In addition, most patients in this study underwent hepatic resection due to malignancy, thus a variety of other tumor-related factors may have contributed to the hypophosphatemia observed postoperatively. It is important to note that increased gene expression of FGF23 can result in elevated fragments without increased protein activity.[23] Although we did not measure intact FGF23 directly, high c-terminal fragments in the absence of intact FGF23 elevation would not cause hypophosphatemia, as seen in our patients. Furthermore, in a previous study on 40 adults with ESLD, serum c-terminal correlated well with intact FGF23 levels[21].

Our patients add the first pediatric data to support FGF23 overproduction in BA and identify hepatocytes as a source for circulating FGF23. Chronic inflammation may play a role in FGF23 elevation as c-terminal FGF23 has been positively associated with inflammatory markers in patients with chronic kidney disease; however, the causal pathway remains unknown.[24] Currently, it is unclear whether FGF23 ectopic expression in hepatocytes is uniquely linked to the syndrome of BA, or whether it is associated with the inflammatory response of hepatocytes to biliary obstruction or progressive fibrosis.

In conclusion, these are the first reported cases of BA associated with increased serum FGF23 due to increased production from diseased liver tissue, resulting in severe hypophosphatemia and hypophosphatemic rickets. To elucidate whether FGF23 is associated with metabolic bone disease and fracture risk in patients with BA[25] and other chronic cholestasis syndromes,[26] further investigations are warranted.

ABBREVIATIONS

BA: biliary atresia
ESLD: end-stage liver disease, FGF23, fibroblast growth factor 23
HHPR: hypophosphatemic rickets
MAS: McCune-Albright syndrome
TRP: tubular resorption of phosphate

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