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Iron Chelation Resulting in Renal Phosphate Wasting

Lynda Cheddani[3], Thierry Leblanc[4], Caroline Silve[5,6,7], Nahid Tabibzadeh[1,2,3], Dominique Prié[8,9], Jean-Philippe Haymann[1,2,3], Marie-Noëlle Péraldi[10], Michel Daudon[1,2,3], Paul Meria[11] and Emmanuel Letavernier[1,2,3]

[1]Sorbonne Université–UPMC Paris 06, UMR S 1155, Paris, France; [2]INSERM, UMR S 1155, Paris, France; [3]Explorations Fonctionnelles Multidisciplinaires, AP-HP, Hôpital Tenon, Paris, France; [4]Service d'Hématologie, AP-HP, Hôpital Saint-Louis, Paris, France; [5]Service de Biochimie et Génétique Moléculaire, AP-HP, Hôpital Cochin, Paris, France; [6]INSERM U1169, Université Paris Sud, Hôpital Bicêtre, Le Kremlin Bicêtre, France; [7]Centre de Référence des Maladies Rares du Métabolisme du Phosphore et du Calcium, Filière de Santé Maladies Rares OSCAR, AP-HP, Paris, France; [8]Paris Descartes Université–Paris 05, Paris, France; [9]Service des Explorations Fonctionnelles, AP-HP, Hôpital Necker, Paris, France; [10]Service de Néphrologie, AP-HP, Hôpital Saint-Louis, Paris, France; and [11]Service d'Urologie, AP-HP, Hôpital Saint-Louis, Paris, France

Correspondence: Emmanuel Letavernier, Service des Explorations Fonctionnelles, Multidisciplinaires, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France. E-mail: emmanuel.letavernier@tnn.aphp.fr

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INTRODUCTION

Acting on fibroblast growth factor receptor 1 (FGFR1), FGF23 reduces renal phosphate reabsorption through downregulation of the sodium phosphate cotransporters NPT2a and NPT2c at the apical membrane of the proximal renal tubule.[1,2]

Human disorders of phosphate handling and hypophosphatemic rickets have been related to increased FGF23 serum levels resulting from mutations affecting the *FGF23* gene itself or genes regulating FGF23 secretion, such as *PHEX* or *DMP1*. These mutations present as autosomal-dominant rickets (ADHR), X-linked dominant rickets (XLHR), and autosomal-recessive hypophosphatemic rickets (ARHR), respectively.[3]

Iron plays a role in FGF23 regulation in humans, and an inverse relationship between iron status and FGF23 concentrations has been described in several populations.[4–6]

Inflammation and iron deficiency increase *fgf23* transcription in mice by activating Hif1α signaling, but relationships between iron and FGF23 secretion in humans remain a matter of debate.[7,8]

Kapelari *et al*. reported an association between iron supplementation and a complete loss of biochemical ADHR phenotype, allowing withdrawal of rickets medication.[6]

Conversely, we report here the case of a man affected by a homozygous *DMP1* rare variant and presenting with hyperphosphaturia associated with high serum FGF23 levels. This exceptional case raises concerns about the relationships between iron chelation and renal phosphate leak, potentially promoting bone demineralization and urolithiasis in genetically predisposed patients.

PATIENT AND METHODS

Clinical Case

A 22-year-old man presented with a medical history of Diamond Blackfan anemia. This rare disorder results from a mutation of *RPS19* gene (RPS19: NM_001022.3; c.320T>G; p.Leu107Arg) responsible for intrinsic progenitor cell defect. There was no consanguinity in the family. He was 150 cm tall, had moderately bowed legs, and weighed 50 kg. Since birth, he had received iterative transfusions inducing post-transfusional hemochromatosis. He had therefore been treated with iron chelators, mainly deferasirox. He progressively developed mild renal failure (serum creatinine 108 μmol/l), and deferasirox was therefore replaced by deferiprone and deferoxamine with doses adapted to serum ferritin levels.

When he was 21 years of age, he developed massive kidney stones treated by reno-ureteroscopy. Stone analysis revealed a predominance of calcium phosphate (mildly carbonated apatite) and, to a lesser extent, the presence of calcium oxalate dihydrate (weddellite).

Initial serum biochemistry findings [normal range] were as follows: calcium, 2.42 [2.16–2.52] mmol/l; phosphate, 0.56 [0.85–1.31] mmol/l; intact parathyroid hormone (PTH), 18 [8–76] pg/ml; 25-hydroxy-vitamin

D (25(OH)D), 45 [30−100] ng/ml, and 1,25[OH]$_2$D, 58 [17−67] pg/ml. Serum bicarbonate level was at the lower limit at 22.5 mmol/l, and potassium level was normal at 3.8 [3.7−5.1] mmol/l. Urine tests revealed a low tubular phosphate reabsorption rate (TPR), 75% [85%−97%] and decreased tubular maximum reabsorption of phosphate to glomerular filtration rate [TmP/GFR] at 0.44 [>0.67] mmol/l.GF. Urine calcium excretion was increased at 7.7 [<6] mmol/d.

The patient therefore presented with biological features associating low serum phosphate level caused by a massive renal loss and hypercalciuria, which was responsible for calcium phosphate nephrolithiasis. These features were consistent with an acquired proximal tubulopathy due to deferasirox. However, the C-terminal FGF23 serum level was 218 [33.7−96.5] RU/ml, suggesting that renal phosphate leak was instead due to increased FGF23 secretion.

A few months later, C-terminal FGF23 decreased transiently to 89 RU/ml, and both serum phosphate and 1,25[OH]$_2$D serum levels increased at the same time up to 0.72 mmol/l and 78 pg/ml, respectively.

Hypotheses and Methods

These observations suggested that renal phosphate loss was due to excessive FGF23 secretion. Therefore, we conducted molecular genetic analysis to detect mutations affecting *FGF23*, *PHEX,* and *DMP1* genes. PolyPhen-2 (available at: http://genetics.bwh.harvard.edu/pph2/, 15/06/2017) and SIFT (available at: http://sift.jcvi.org/, 15/06/2017) software was used to predict *in silico* whether an amino acid substitution would affect protein function. Informed consent was obtained from the patient and both parents to perform DNA analyses and to collect clinical and biochemical data.

Moreover, the simultaneous fluctuations of FGF23 and phosphate levels supported the hypothesis that intermittent determinants, for example, drugs, would be involved. Considering *in vitro* and animal studies suggesting that iron deficiency can increase FGF23 levels, the contribution of iron chelators in renal loss of phosphate has been hypothesized.[7,8] Actually, this patient had intermittent iron chelator intake. To test this hypothesis, iron chelators were withdrawn for 72 hours. C-terminal FGF23 and phosphate levels were monitored during iron chelation, after discontinuation for 72 hours, and after treatment reintroduction several weeks later.

RESULTS

Molecular Genetic Analysis

DNA sequencing of DMP1 gene (NM_004407) revealed a rare homozygous variant affecting exon 5 (c.427C>A, p.Gln159Lys). No mutation has been identified in PHEX or FGF23 genes. The Gln159Lys variant is predicted to be deleterious. Both parents were heterozygous carriers of this rare variant.

Metabolic Response to Iron Chelation Withdrawal and Reintroduction

After discontinuation of iron chelators for 72 hours, C-terminal FGF23 levels values halved and serum phosphate levels normalized from 0.56 to 0.89 mmol/l (Figure 1). Stopping iron chelation during 72 hours induced resolution of renal phosphate loss. Treatment reintroduction had the opposite effect, and a decline in serum phosphate level was observed (back to 0.56 mmol/l), due to an increase in C-terminal FGF23 levels (116.6 to 276.4 RU/ml). These results provided evidence that iron chelation promoted renal loss of phosphate, through an increase in FGF23 levels (Figure 1).

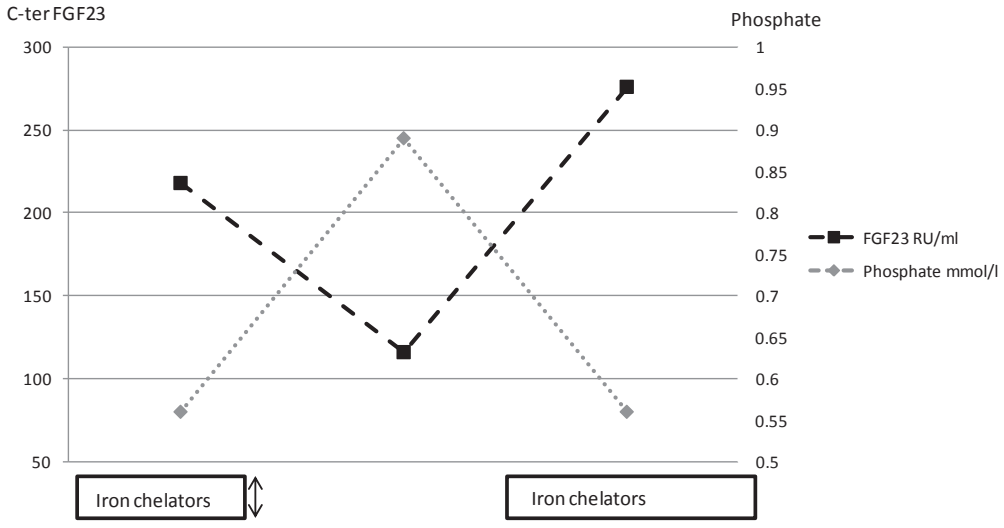

Figure 1. Evolution of serum phosphate and C-terminal FGF23 levels under deferiprone and deferoxamine, 72 hours after stopping iron chelation, and after iron chelator reintroduction. Dotted lines indicate serum phosphate–level evolution; dashed lines correspond to C-terminal FGF23 serum-level evolution.

DISCUSSION

To our knowledge, this is the first report of an association between iron chelation in a human and so-called hypophosphatemic rickets. Farrow *et al*. previously demonstrated *in vitro* that iron chelation with deferoxamin increased FGF23 mRNA expression in osteoblasts by 20-fold.[7] David *et al*. provided evidence that functional iron deficiency, following hepcidin injection into wild-type mice, stimulated FGF23 production and preferentially increased circulating concentrations of C-terminal FGF23.[8]

Regarding the patient, we first hypothesized that renal phosphate loss resulted from an acquired proximal tubulopathy induced by deferasirox.[9] Actually, nephrotoxicity is the most frequent adverse effect of deferasirox treatment. Nephrotoxicity can present as an acute or chronic decrease in glomerular filtration rate, and features of proximal tubular dysfunction are also frequent.[10] High levels of FGF23 rapidly ruled out this assumption. Genetic analysis identified a rare homozygous *DMP1* variant, but the pathogenicity of this variant is unknown to date. It seems likely that the presence of both minor alleles may predispose to renal phosphate leak through an increase in FGF23; however, in the absence of iron chelation, biological expression was minimal. It appears that the *DMP1* variant was a predisposing condition requiring a second hit to promote renal phosphate leak. The exact role of DMP1 remains unknown, but recent murine experimental models have shown that mutations in the *DMP1* gene create a lower set point for extracellular phosphate and maintain it through the regulation of FGF23 cleavage and expression.[11]

In this case, chronic medical iron chelation played a major role in the ARHR biochemical phenotype by simulating a transitory iron deficiency. This hypothesis was confirmed by serum phosphate levels and C-terminal FGF23 levels evolution associated with iron chelator withdrawal and reintroduction.

Previous studies reported an association between iron status and FGF23 levels. Imel *et al*. observed an inverse correlation between iron status and (i) C-terminal FGF23 levels in healthy individuals and (ii) both intact and C-terminal FGF23 levels in patients presenting with ADHR (*FGF23* mutation) or XLH (*PHEX* mutation).[4] Iron substitution has been associated with a C-terminal FGF23 level reduction, and with phosphate renal loss reduction in ADHR patients.[4–6] In this case, C-terminal FGF23 was dramatically increased, a further argument in favor of relative iron "deficiency."

Beyond this case, another issue of interest concerns nephrolithiasis composition (calcium phosphate). Recently, Wong *et al*. reported that deferasirox was associated with a high prevalence of nephrolithiasis and bone demineralization in patients affected by thalassemia.[12] In this study, among patients receiving deferasirox, those affected by nephrolithiasis had significantly lower serum phosphate and ferritin levels. FGF23 was not assessed, but it might be hypothesized that deferasirox would promote renal phosphate leak and calcium phosphate stones in genetically predisposed individuals. More recently, Wong *et al*. observed hypercalciuria in 92% of subjects on deferasirox, with a significant dose-dependent relationship, but also in 83.4% of the smaller group of subjects on deferoxamine.[13] In the present report, the patient was also affected by hypercalciuria; however, the role of iron chelators in tubular defects leading to hypercalciuria remains unknown.

In conclusion, this case presentation supports the hypothesis that iron status would be involved in C-terminal FGF23 and serum phosphate level regulation. Iron chelation was associated with increased C-terminal FGF23 and decreased serum phosphate levels, which were rapidly reversible after stopping treatment, in a genetically predisposed individual. Further studies are required to identify the mechanisms involved, in particular the sequence of events modifying FGF23 secretion and cleavage.

Beyond this case, the role of iron chelators in kidney stone formation, osteomalacia, and bone demineralization is an open question that must be addressed.

DISCLOSURE

All the authors declared no competing interests.

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