Resolution of severe, adolescent-onset hypophosphatemic rickets following resection of an FGF-23-producing tumour of the distal ulna

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Abstract

Oncogenic hypophosphatemic osteomalacia (OHO) is an uncommon hypophosphatemic syndrome characterized by bone pain, proximal muscle weakness and rickets. It has been postulated that OHO results from overproduction of a humoral phosphaturic factor by an occult tumour. Recently, some OHO tumours have been shown to elaborate fibroblast growth factor-23 (FGF-23), which causes renal phosphate wasting when administered to mice. The purpose of this study was to undertake detailed investigations to confirm the diagnosis of OHO in a pediatric patient and to document the biochemical, radiographic and bone histological phenotype before and after tumour removal. We describe an 11-year-old, previously healthy girl with significant pain and functional disability associated with hypophosphatemic rickets. Circulating 1,25-(OH)2 vitamin D was very low (14 pM; N: 40–140) while the FGF-23 serum level was markedly elevated [359.5 reference units (RU)/ml, N: 33–105]. An iliac bone biopsy revealed severe osteomalacia, but periosteocytic lesions, as are typical for X-linked hypophosphatemic rickets, were not seen. Sequence analyses of the PHEX and FGF23 genes were normal. A radiographic skeletal survey revealed a small exostosis of the left, distal ulnar metaphysis. A tumour was subsequently removed from this site and the pathology was consistent with benign, fibro-osseous tissue. Serum FGF-23 was normal when measured at 7 h post-operatively, while serum phosphate reached the low-normal range at 16 days following surgery. An iliac bone biopsy taken 5 months after the operation showed improvement, but not yet resolution, of the osteomalacia. Biochemical parameters of bone and mineral metabolism suggested that complete resolution of the osteomalacia was not achieved until 12 months following surgery. One year after tumour removal, the patient was pain-free and had resumed a normal level of activity. The rapid normalization of FGF-23 levels following removal of a benign tumour and the subsequent improvement in the biochemical and histological parameters of bone and mineral metabolism suggest that FGF-23 played a key role in this girl’s disease.

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Introduction

Oncogenic hypophosphatemic osteomalacia (OHO) is an acquired, paraneoplastic syndrome that results in markedly deranged mineral and skeletal metabolism. The disorder is characterized by hypophosphatemia due to renal phosphate wasting, osteomalacia, bone pain, proximal muscle weakness, fractures and functional disability. A very low circulating 1,25-(OH)2 vitamin D level despite hypophosphatemia is the biochemical hallmark of the disease [4]. OHO is commonly associated with small, slow-growing tumours of mesenchymal origin that may be difficult to detect [4]. These tumours are thought to produce a circulating phosphaturic factor [6]. If the causative tumour can be located and completely removed, there is normalization of serum phosphate and remission of the bone disease. OHO is an uncommon entity in children, with fewer than 20 pediatric cases reported in the literature to date [4,21].

OHO shares similarity with two genetic diseases, X-linked hypophosphatemia (XLH) and autosomal domi-
nant hypophosphatemic rickets (ADHR), and may be difficult to distinguish from the genetic hypophosphatemias on clinical grounds. XLH results from mutations in the PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) gene [11], which encodes a membrane-bound endopeptidase, whereas ADHR is associated with mutations in the FGF23 gene, which encodes a phosphaturic factor by the same name [1].

Here we report the case of an 11-year-old girl with severe OHO due to an inconspicuous, fibro-osseous neoplasm and describe the clinical, biochemical and bone histological course of the disease before, and up to 12 months following, excision of the tumour. We further provide evidence for the role of fibroblast growth factor-23 (FGF-23), a circulating phosphaturic factor[18,19], in the pathogenesis of this patient’s disease.

Clinical report

The patient, a girl of French-Canadian/Scottish descent, first came to medical attention at 11 years of age, when she complained of bilateral knee pain that had gradually developed over a 2-year period. Previously she had been a healthy, elite soccer player with a high level of physical activity. An antalgic gait was noted but no specific diagnosis was made.

By 11.5 years of age, she was wheelchair bound due to significant pain in the hips and knees. At this time, her height was at the 50th percentile, and she was in mid-puberty (Tanner stage III overall). Radiographs of the wrists and knees showed signs of rickets. A Tc99m MDP bone scan revealed numerous foci of increased uptake (mid-humeral shafts, right femoral neck, proximal and mid-right femoral shaft, leftibia, ribs and left sacroiliac joint), which correlated with Looser’s Zones on plain radiographs. An extensive evaluation for suspected malignancy was negative. However, low levels were found for serum inorganic phosphorus (0.6 mM; N: 1.0–1.7 mM), the threshold maximum for renal tubular phosphate reabsorption/glomerular filtration rate (TmP/GFR, 0.25 mM; N: 0.78–1.94) and serum 1,25-(OH)2 vitamin D (14 pM; N: 40–140), whereas serum levels of ionized calcium, intact PTH and 25-OH vitamin D were normal. Serum alkaline phosphatase was elevated (631 U/l; N: 105–420). There was no biochemical evidence of a generalized tubulopathy.

A diagnosis of hypophosphatemic rickets was made and treatment was initiated with calcitriol (Rocaltrol, Roche; 0.25 μg twice daily), and sodium acid phosphate (Phosphate-Novartis, Novartis Pharmaceuticals; 500 mg of elemental phosphorus four times daily). Subsequently, her pain improved somewhat and she was able to walk independently, albeit slowly and with a limp.

At 13 years of age, a diagnosis of OHO was suspected, given the ongoing pain and disability despite medical therapy. A radiographic skeletal survey revealed an ill-defined protuberant lesion of the left distal ulnar metaphysis (Fig. 1A), which was confirmed by magnetic resonance imaging and computed tomography (Fig. 2). At the age of 14.8 years, medical therapy was withdrawn and the tumour was removed surgically 1 week later. Detailed investigations were undertaken to confirm the diagnosis of OHO and to chart the changes before and after tumour removal.
Methods

**PHEX and FGF23 genetic analyses**

Since the biochemical features of OHO resemble those of ADHR and XLH, PHEX and FGF23 mutation analyses were undertaken. For analysis of the PHEX gene, genomic DNA was extracted from anticoagulated blood using QUlamp kits (Qiagen). The coding sequences and the flanking sequences of the 22 exons of the PHEX gene were amplified using Taq DNA polymerase (Perkin-Elmer-Cetus) and series of specific oligonucleotide primers [10]. The presence of mutations was screened by heteroduplex analysis of PCR products [22], and was confirmed by nucleotide sequence analysis using Big Dye Terminator cycle sequencing and an ABI prism 3100 DNA sequencer.

![Graphs showing serum levels](image)

Fig. 3. Serum ionized calcium (a), phosphate (b), alkaline phosphatase (c), intact PTH (d), 1,25-dihydroxyvitamin D (e) and TmP/GFR (f) levels before surgery, and up to 1 year following resection of the FGF-23-producing ulnar tumour. (g) Serum FGF-23 levels pre- and post-operatively, showing rapid normalization of FGF-23 following removal of the tumour.
FGF23 mutation analysis was undertaken in the following manner. The three FGF23 exons, including the intron–exon splice junctions, were PCR-amplified with intronic primers: (Exon 1 Forward: AATCTCAGCACCAGCTC, Reverse: GATGGACAACAGGTGCTC; Exon 2 Forward: TTTCAGGAGGTGCTTGAAGG, Reverse: TTGCAAATGGTGACCAACAC; and Exon 3 Forward: CTTCAGTGGTTCGCTCTTG, Reverse: TGCTGAGGGATGGGTTAAAG) using 20 ng of genomic DNA as templates. PCR conditions for all experiments were: 1 min 95°C, followed by 35 cycles of 1 min 95°C, 1 min 57°C, 1 min 72°C, and a final extension of 7 min at 72°C. Amplified exons were analyzed by DNA sequencing with the appropriate forward primers using the ThermoSequenase Kit (USB; Cleveland, OH) and direct incorporation of [33P]dideoxynucleotides. Sequences were resolved on 6% acrylamide gels and autoradiography was performed.

Biochemistry

Serum FGF-23 concentrations were evaluated with a commercially available assay (Human FGF-23 C-Terminal Elisa Kit, Immutopics, San Clemente, CA) according to the manufacturer’s instructions. This is a two-site sandwich ELISA that recognizes the C-terminal portion of FGF-23. Results were compared to published pediatric reference data [12]. Serum and urine concentrations of calcium, phosphorus and creatinine as well as serum alkaline phosphatase activity were measured using standard methods. Serum intact parathyroid hormone was determined by immunoradiometric assay (N-tact*, Incstar Corp., Stillwater, MN). 25-OH vitamin D and 1,25-(OH)2 vitamin D were measured with radioimmunoassays (25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Osteo SP; Incstar Corp.). All samples were obtained after fasting, and all urine studies except those in the immediate post-operative period were obtained from the second void sample in the morning.

Bone densitometry

Lumbar spine (L2-4) densitometry was performed using a Lunar Prodigy device (Lunar Corp., General Electric; Madison, WI). Bone mineral apparent density was determined according to the method proposed by Kroger et al. [13]. Results were transformed to age- and sex-specific Z-scores using published reference data [20].

Histomorphometry

Full-thickness transiliac bone biopsies were obtained on the 5th day after dual tetracycline labeling (Declomycin, Wyeth-Ayerst Canada Inc., Montréal, Canada) and analyzed as described previously [9]. Quantitative histomorphometric results were compared to reference data as established by this laboratory [9].

Results

A lesion measuring 2.0 cm × 1.2 cm × 0.8 cm was removed from the distal ulna. Pathological examination revealed non-specific fibro-osseous tissue, without evidence of malignancy. Tumour tissue was not available for further studies. At 5 months post-operatively, the patient walked...
with a normal gait and had resumed low-level physical activity. She was pain-free by 12 months after the operation and was able to engage in her usual sport activities.

Sequence analyses of the \textit{PHEX} and \textit{FGF23} genes revealed no mutations. Peri-operative biochemical analyses are presented in Figs. 3a–f. Serum phosphate rose to the lower limit of the reference range by 16 days following surgery. The alkaline phosphatase initially rose following tumour removal, before undergoing a gradual decline. Normalization of the alkaline phosphatase was not achieved until 12 months following surgery. Intact PTH rose to a peak at 3 weeks following surgery, and normalized by 5 months after tumour excision. At 24 h post-operatively, serum 1,25-(OH)2 vitamin D rebounded to 9-fold above the immediate pre-operative value, and declined to the upper limit of normal by 12 months after surgery. Serum FGF-23 was significantly elevated before tumour excision [359.5 reference units (RU)/ml; \textit{N}: 33–103], with normalization when measured at 7 h post-surgery (Fig. 3g). FGF-23 remained within the normal range upon re-evaluation 5 months later.

Bone mineral apparent density at L2-4 increased from 0.266 g/cm3 pre-operatively to 0.350 g/cm3 at 5 months after the operation, corresponding to an increase in \textit{Z}-score from \(-2.5\) to \(-0.8\). Twelve months following surgery, there was fusion of the growth plates with correction of the mineralization defect (Fig. 1B).

An iliac bone specimen obtained 8 months before tumour excision showed severe osteomalacia, with a large amount of osteoid and poor uptake of dual-tetracycline labels. There was no evidence of periosteocytic lesions, the histological hallmark of XLH.

Five months following excision of the tumour, osteoid indices had markedly decreased and there was elevated bone formation activity (Fig. 4 and Table 1).

### Discussion

We describe an adolescent girl with severe OHO due to a small, histologically benign tumour that secreted high levels of FGF-23. OHO may be difficult to distinguish from XLH and ADHR on clinical and biochemical grounds. However, the disease should be suspected in the pediatric patient with hypophosphatemic rickets when there is significant pain and weakness, a negative family history of hypophosphatemia, and a very low 1,25-(OH)2 vitamin D level. Although our patient’s normal height and straight limbs were also suggestive of an acquired process, short stature and skeletal deformity are not universal in ADHR and XLH [7,8]. Both ADHR and XLH may not manifest until the late- or post-pubertal years, and may be associated with a mild phenotype [7,8,16]. In this situation, evaluation of the \textit{FGF23} and \textit{PHEX} genes may provide the diagnostic clue. OHO can be further distinguished from XLH on the basis of bone histological findings, as periosteocytic lesions are commonly present in XLH, but not in other forms of osteomalacia [14]. These periosteocytic lesions are halos of unmineralized bone surrounding osteocyte lacunae and are thought to indicate a primary osteoblast defect in XLH [5,14].

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before tumour removal (age 14.0 years)</th>
<th>Percent of age-matched mean* (before tumour removal)</th>
<th>After tumour removal (age 15.2 years)</th>
<th>Percent of age-matched mean* (after tumour removal)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural parameters</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Core width (mm)</td>
<td>8.7</td>
<td>122.5</td>
<td>7.4</td>
<td>86.0</td>
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<td>Cortical width ((\mu)m)</td>
<td>1002.0</td>
<td>111.7</td>
<td>1567.0</td>
<td>133.0</td>
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<tr>
<td>Bone volume/tissue volume (%)</td>
<td>24.7</td>
<td>101.2</td>
<td>29.8</td>
<td>116.0</td>
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<td>Trabecular thickness ((\mu)m)</td>
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<td>116.2</td>
<td>299.0</td>
<td>190.4</td>
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<td>Trabecular number ((\mu)m)</td>
<td>1.4</td>
<td>82.4</td>
<td>1.0</td>
<td>62.5</td>
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<td><strong>Formation parameters</strong></td>
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<tr>
<td>Osteoid thickness ((\mu)m)</td>
<td>36.4</td>
<td>543.3</td>
<td>12.7</td>
<td>201.6</td>
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<tr>
<td>Osteoid surface/bone surface (%)</td>
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<td>435.5</td>
<td>62.0</td>
<td>238.5</td>
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<td>Osteoid volume/bone volume (%)</td>
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<td>5.1</td>
<td>232.0</td>
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<td>Mineralizing surface/bone surface (%)</td>
<td>**</td>
<td>**</td>
<td>57.7</td>
<td>461.6</td>
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<td>Mineral apposition rate ((\mu)m/day)</td>
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<td>**</td>
<td>0.98</td>
<td>121.0</td>
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<td>Bone formation rate/bone surface ((\mu)m3/(\mu)m2/year)</td>
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<td>**</td>
<td>207.0</td>
<td>559.5</td>
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<td>Mineralization lag time (day)</td>
<td>**</td>
<td>**</td>
<td>13.9</td>
<td>90.8</td>
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<td><strong>Resorption parameters</strong></td>
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<tr>
<td>Eroded surface/bone surface (%)</td>
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<td>34.7</td>
<td>20.1</td>
<td>111.7</td>
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<tr>
<td>Osteoclast surface/bone surface (%)</td>
<td>0.2</td>
<td>22.2</td>
<td>0.8</td>
<td>82.7</td>
</tr>
</tbody>
</table>

*Mean values according to Ref. [9].
**Unable to calculate due to severe osteomalacia, resulting in poor uptake of tetracycline label.
differential diagnosis. In OHO, however, FGF-23 levels can be expected to normalize as early as 30 min following removal of the offending tumour [23], allowing for rapid verification of complete surgical excision. Serial FGF-23 measurements may prove useful in the future, for long-term monitoring of disease recurrence. Indeed tumour recurrence has been reported up to 17 years after initial removal of an OHO lesion [3]. In our patient, while FGF-23 was rapidly cleared following tumour removal, serum phosphate did not normalize until 16 days after surgery. The reason for the relatively slow normalization of serum phosphate following tumour removal is unclear.

A low serum 1,25-(OH)2 vitamin D level is the biochemical signature of OHO, a phenomenon which occurs despite hypophosphatemia [4]. Extracts of OHO tumours have been shown to inhibit the production of 1,25-(OH)2 vitamin D [2], through suppression of renal 1-alpha-hydroxylase activity [15], which may be a direct effect of FGF-23 [17]. The significant rise in 1,25-(OH)2 vitamin D following tumour removal and normalization of FGF-23 are consistent with this hypothesis. However, it remains unclear why OHO patients do not manifest hypocalcaemia in the presence of depressed 1,25-(OH)2 vitamin D levels. Our patient’s precipitous rise in PTH post-operatively corresponded with the time at which the serum phosphate had reached the lower limit of normal. This observation is consistent with the hypothesis that transient hyperparathyroidism following OHO tumour excision results from disinhibition of PTH secretion following resolution of the hypophosphatemia.

Biochemical parameters of bone metabolism correlated with our patient’s clinical symptomatology, as she reported resumption of a normal lifestyle once the alkaline phosphatase level approached the upper limit of normal. Mineralization of osteoid was directly documented by a decrease in osteoid indices and indirectly by the rapid increase in bone mineral apparent density. This was accompanied by normalization of the mineralization lag time and vigorous bone formation activity. The potential for restitution of mineralized skeletal tissue following excision of an OHO tumour during youth is reflected in the 31% increase in spinal bone mineral apparent density that occurred during the 5-month post-operative period.

In summary, we describe a teenage girl with severe OHO who experienced cure following excision of a benign, fibrousosseous lesion. This case highlights the importance of considering OHO in the differential diagnosis of children and adolescents who present with hypophosphatemic syndromes. While serum phosphate normalized by 16 days following tumour removal, biochemical and histological parameters of bone and mineral metabolism suggested that complete reversal of the mineralization defect was not achieved until 1 year post-operatively. The rapid normalization of FGF-23 following removal of the OHO tumour suggests that FGF-23 played a key role in the pathogenesis of this patient’s disease.

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