CASE REPORT

Oncogenic hypophosphataemic osteomalacia: biomarker roles of fibroblast growth factor 23, 1,25-dihydroxyvitamin D₃ and lymphatic vessel endothelial hyaluronan receptor 1

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Abstract

Oncogenic osteomalacia (OOM) is characterised by tumour production of fibroblast growth factor 23 (FGF23) that results in hypophosphataemia and renal phosphate wasting, reduced 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) synthesis and osteomalacia. Here, we demonstrate the roles of serum FGF23 and 1,25(OH)₂D₃, together with the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), as biomarkers for OOM. A previously well 52-year-old man presented with a 2-year history of generalised musculoskeletal pain and proximal myopathy. He had hypophosphataemia, elevated serum alkaline phosphatase activity, low serum 1,25(OH)₂D₃ and a reduced tubular maximum of phosphate/glomerular filtration rate. These findings indicated a diagnosis of OOM, but magnetic resonance imaging (MRI) and octreotide scintigraphy did not identify any tumours. Treatment with oral phosphate and calcitriol resolved the symptoms and biochemical abnormalities within 6 months. Four years later, he relapsed whilst on treatment with oral phosphate and calcitriol. Serum FGF23 concentration was elevated and MRI identified a 2 cm tumour within Hoffa's fat pad of the left knee. Removal of the tumour resulted in a complete resolution of symptoms and normalisation of the serum biochemical abnormalities including serum FGF23. Histology demonstrated a phosphaturic mesenchymal tumour, mixed connective tissue variant (PMTMCT), which revealed immunostaining with anti-LYVE-1 antibody and hence the presence of lymphatic vessels. Serum FGF23 and 1,25(OH)₂D₃ were found to be reliable biomarkers for OOM. In addition, the demonstration of lymphatics in the PMTMCT helps to distinguish this tumour from most typical benign haemangiomas.

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Introduction

Oncogenic osteomalacia (OOM) is caused by small, often difficult to locate tumours, most frequently phosphaturic mesenchymal tumours, mixed connective tissue variants (PMTMCTs), which may occur anywhere in the body (1). These tumours are usually benign but over 10% of PMTMCTs may recur or produce metastases. This acquired form of osteomalacia is associated with hypophosphataemia, hyperphosphaturia, low circulating 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) concentrations and elevated serum fibroblast growth factor 23 (FGF23) concentrations (2). OOM tumours have been shown to contain high levels of FGF23 mRNA and protein (1), and i.v. infusions of FGF23 have been shown to inhibit renal phosphate reabsorption and 1,25(OH)₂D₃ synthesis in mice (3). In this study, we have evaluated the use of serum 1,25(OH)₂D₃, FGF23 and expression of the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) as biomarkers for a PMTMCT.

Case report

A previously well 52-year-old man presented with a 2-year history of progressive fatigue, generalised severe musculoskeletal pain and difficulty in walking. There was no history of weight loss, fractures, joint stiffness, alcohol excess, corticosteroid use, dietary deficiency of calcium or vitamin D, malabsorption, thyrotoxicosis, exposure to heavy metals or degraded antibiotics, or malignancy. He was a non-smoker. In addition, there was no family history of metabolic bone disease. Clinical examination revealed a proximal myopathy. He was clinically euthyroid and not Cushingoid. Bone and joint deformities or cutaneous haemangiomas were not present. His height was 1.83 m, and he had a normal body mass index of 24.5 kg/m². On investigation, he had hypophosphataemia, an elevated serum alkaline phosphatase activity, a low serum 1,25(OH)₂D₃ and a reduced tubular maximum of phosphate/glomerular

filtration rate (TmP/GFR) of 0.4 mmol/l (normal range: 0.8–1.35 mmol/l; Fig. 1). Serum calcium, creatinine, PTH and 25-hydroxyvitamin D₃ (25OHD₃) were normal and there was no glycosuria or aminoaciduria. These findings indicated a diagnosis of OOM (2). However, whole-body magnetic resonance imaging (MRI) and octreotide scintigraphy did not identify any tumours. Treatment with oral phosphate and calcitriol was commenced. His biochemical abnormalities and symptoms resolved within 6 months (Fig. 1). Follow up with

an annual whole-body MRI did not identify the causative tumour.

Four years later, despite treatment, he relapsed. The serum FGF23 concentration (4, 5) was elevated (Fig. 1) and on this occasion MRI identified a 2 cm tumour of the left knee (Fig. 2), removal of which was associated with marked clinical improvement and normalisation of the serum biochemical abnormalities including serum FGF23 (Fig. 1). Histology revealed a haemangioma-like PMTMCT that contained lymphatic vessels; these were identified by expression of the lymphatic endothelial cell marker LYVE-1 (6) (Fig. 3). A definable geniculate artery supplying the tumour was not observed to be present. After 30 months, he remains well and asymptomatic, with no medication.

Discussion

The biochemical features of OOM include hypophosphataemia, elevated serum alkaline phosphatase activity, reduced TmP/GFR, low or inappropriately decreased circulating 1,25(OH)₂D₃ concentrations and increased serum concentrations of FGF23 (2, 4, 5). These are similar to those found in the hereditary forms of metabolic bone disease such as X-linked hypophosphataemic rickets (XLHR) and autosomal dominant hypophosphataemic rickets (ADHR) (4) (Fig. 4). While the clinical and biochemical abnormalities are lifelong in ADHR and XLHR, they resolve after removal of the PMTMCT in OOM (2, 4, 5), as illustrated by this patient. However, the similarities between OOM, ADHR and XLHR indicate that they may involve the same phosphate-regulating pathway (2). Indeed, this is the case, as OOM tumours express FGF23 and the phosphate regulating endopeptidase on the X chromosome (PHEX) (7), mutations of which cause ADHR and XLHR, respectively (4). An elevated serum FGF23 concentration and an inappropriately low circulating 1,25(OH)₂D₃ concentration in combination with hypophosphataemia are useful biomarkers for the detection of OOM (2, 4, 5). In one study, all 13 OOM patients with confirmed tumours had elevated serum FGF23 concentrations (5). Serum FGF23 is also raised in the inherited forms of hypophosphataemic rickets, XLHR and ADHR, and in renal failure (4, 5). However, in renal failure, the elevated serum FGF23 is associated with either a normal or increased serum phosphate. The measurement of serum FGF23 is also of use in monitoring the response to successful surgery, with circulating concentrations decreasing to normal values following tumour resection (Fig. 1) (4), as illustrated by this report.

FGF23, a 251 amino acid secreted peptide that is predominantly expressed in bone, is an important regulator of phosphate and vitamin D homeostasis (4) (Fig. 4). For example, mice lacking FGF23 have elevated serum phosphate concentrations and increased circulating 1,25(OH)₂D₃ concentrations as well as an abnormal bone phenotype (8). In contrast, the exogenous administration

Figure 1 Biochemical findings over 6 years: (A) at presentation, (B) on treatment with phosphate and calcitriol, (C) relapse of symptoms, (D) pre- and (E) post-surgical excision of tumour. Reference ranges are indicated by horizontal broken lines. Fibroblast growth factor 23 (FGF23) estimations only became available during year 4.

Figure 2 MRI of the left knee. (A) Sagittal proton density fat-saturated image demonstrating a rounded lesion (arrows) of increased signal intensity in Hoffa's fat. There are some small foci of decreased signal within the lesion corresponding to areas of calcification at histology. (B) Axial T1-weighted image demonstrating a well-defined rounded mass (arrows) in Hoffa's fat which is of intermediate/low signal intensity.

Figure 3 Histology of the PMTMCT. (A) Haematoxylin–eosin staining showed a well-vascularised tumour containing spindle-shaped mesenchymal cells and bone trabeculae (×200 magnification). (B) Immunostaining with peroxidase and anti-LYVE-1 antibody revealed LYVE-1 expression in vessel endothelium (×400 magnification).

of FGF23 in mice leads to decreased phosphate reabsorption by the proximal renal tubule, by inhibiting the sodium–phosphate co-transporters, types IIa and IIc (NPT2a and NPT2c) (3, 9). FGF23 administration also inhibited the synthesis of $1,25(\text{OH})_2\text{D}_3$ by reducing the renal expression of 25-hydroxyvitamin D-1 α -hydroxylase (3). This inhibition of the renal 25-hydroxyvitamin D-1 α -hydroxylase by FGF23 provides an explanation for the observed inappropriately low serum concentrations of $1,25(\text{OH})_2\text{D}_3$ in patients with hypophosphataemic OOM: hypophosphataemia would normally be expected to lead to elevated serum $1,25(\text{OH})_2\text{D}_3$ concentrations as this increases renal 25-hydroxyvitamin D-1 α -hydroxylase activity (2). Thus, an inappropriately low serum $1,25(\text{OH})_2\text{D}_3$ concentration in association with hypophosphataemia is a reliable biomarker for OOM, but is not specific, as low serum $1,25(\text{OH})_2\text{D}_3$ concentrations are also typically found in patients with XLHR and ADHR (2).

Most tumours causing OOM are of mesenchymal origin and have been reported to be haemangiomas, haemangiopericytomas, osteo- or chondrosarcomas and

giant cell tumours (1). However, more than 80% of OOM mesenchymal tumours share a distinct histological appearance, consistent with that of a PMTMCT (1). These tumours are composed of spindle-shaped neoplastic cells, prominent blood vessels, microcysts, osteoclast-like giant cells, cartilage-like matrix and bone (1). Most PMTMCTs are benign with <13% being recurrent or metastatic (1). Lymphatic vessels are present in very few vascular tumours, most notably lymphangiomas (6). Histology in this case revealed that the PMTMCT was haemangioma-like and that unlike typical benign haemangiomas it contained lymphatic vessels; these were identified by the expression of lymphatic endothelial cell marker LYVE-1 (6) (Fig. 3). LYVE-1 expression has previously been reported to occur in Kaposi's sarcoma and angiosarcomas, but has been observed to be absent in haemangiopericytomas, and capillary, cavernous and epithelioid haemangiomas (6). Our finding of LYVE-1 expression in the tumour (Fig. 3) associating with OOM represented the first observation of lymphatic vessels in a PMTMCT, a feature which

Figure 4 Role of fibroblast growth factor 23 (FGF23) in phosphate homeostasis and oncogenic osteomalacia (OOM), X-linked hypophosphataemic rickets (XLHR) and autosomal dominant hypophosphataemic rickets (ADHR). Under physiological conditions, FGF23 is secreted predominantly by bone and undergoes degradation by the phosphate regulating endopeptidase homolog on the X chromosome (PHEX) and other proteolytic enzymes. FGF23, which mediates its actions via an FGF receptor and a likely co-receptor (Klotho), acts on renal proximal tubular (PT) cells to decrease the expression of the sodium–phosphate co-transporters NPT2a and NPT2c, and thereby reduce phosphate reabsorption. Increased amounts of FGF23 which may result from increased production or decreased degradation, will lead to urinary phosphate wasting and hypophosphataemia. Increased production of FGF23 occurs in OOM, in which PMTMCTs secrete FGF23 at levels that are several hundred fold greater than normal (thickened arrow). This overwhelms the degradation pathway and leads to inhibition of renal phosphate excretion and $1,25(\text{OH})_2\text{D}_3$ synthesis, with resulting phosphaturia and reduced intestinal absorption of calcium and phosphate respectively. The combined loss of phosphate and decreased concentration of $1,25(\text{OH})_2\text{D}_3$ lead to hypophosphataemia and osteomalacia. Decreased degradation of FGF23 occurs in XLHR and ADHR, which are due to mutations of the *PHEX* and *FGF23* genes respectively. Thus, in XLHR, *PHEX* mutations lead to an inactivity of PHEX, whilst in ADHR the mutant FGF23, which is still active, is resistant to cleavage. The resulting excess FGF23 leads to phosphaturia.

clearly distinguishes this tumour from most vascular tumours. Indeed, we have subsequently confirmed this finding in eight other PMTMCs (10).

OOM is an acquired disorder due to usually benign mesenchymal tumours secreting humoral factors such as FGF23, which inhibit renal tubular reabsorption of phosphate and impair the synthesis of 1,25(OH)₂D₃ (2, 4, 5). Medical treatment with oral phosphate and calcitriol is generally effective in ameliorating the pain, myopathy, osteomalacia and fractures (2). In some patients with OOM, oral phosphate therapy may occasionally be insufficient, and parenteral phosphate therapy may be required. However, some patients may relapse despite medical therapy. Moreover, the medical therapy may itself be associated with long-term complications such as hypercalcaemia, hypercalciuria, renal stones, renal failure and secondary or tertiary hyperparathyroidism (2). Thus, the optimal treatment for OOM is surgical removal of the tumour, which results in a prompt improvement of the symptoms, and resolution of the biochemical abnormalities and remineralisation of the bone (2). However, these tumours are difficult to find as they are often small and can occur anywhere in the body (11). Hence, pre-operative localisation is essential and a number of imaging modalities such as computerised tomography (CT) scans or MRI, or somatostatin receptor scintigraphy or positron emission with CT (PET-CT) have been utilised with varying success (11–14). CT scanning and MRI have been widely utilised for the detection of OOM tumours, and MRI is preferred for the detection of bone and soft tissue lesions (12). However, these conventional imaging methods frequently fail to identify the small OOM tumours, and other methods have been investigated to facilitate the detection of such occult tumours (11–14). *In vitro* studies have revealed that many OOM tumours express the somatostatin subtype 2 (sst2) receptor (15) and octreotide scintigraphy has been used to successfully detect OOM tumours in 18 cases (11, 16–18). The success rate of detecting OOM tumours by octreotide scintigraphy in one study of seven patients has been reported to be >70% (11). In addition, PET-CT imaging is proving to be useful in the detection of OOM tumours (13, 14). PET most commonly employs F-18 fluorodeoxyglucose (F-18 FDG) as its radioactive tracer and F-18 FDG PET-CT has been used to localise an OOM tumour (13). However, the uptake of F-18 FDG correlates with the proliferative activity of the tumour (19), and as OOM tumours are generally benign and slow-growing (1), it seems unlikely that F-18 FDG PET-CT will be of great utility for OOM tumour detection. The use of Ga-68 DOTA-NOC, a modified octreotide molecule with a higher affinity for sst2 receptors than existing radiolabelled somatostatin analogues, as the PET-CT radioactive tracer, has been highly successful in localising the tumour in one OOM patient (14). The use of radiolabelled somatostatin analogues appears promising but needs to be further

evaluated for the detection of OOM tumours. The finding of sst2 receptors on OOM tumours (11, 17, 18) has not only helped in their detection but also in treatment. Thus, the use of s.c. octreotide has been shown to completely inhibit renal phosphate wasting in one patient with OOM (17) and to partially improve phosphate homeostasis in two other patients (16, 18). In summary, surgical removal of OOM tumours remains the treatment of choice (2), but there are challenges in locating these small and occult tumours that require the use of multiple imaging modalities (11–14). During the period of these investigations, which could be several years, medical treatment with oral phosphate and calcitriol, will help to ameliorate the symptoms and biochemical abnormalities (2). The use of s.c. somatostatin analogues may also be beneficial in those patients whose OOM tumour expresses sst2 receptors and is detectable by octreotide scintigraphy (17).

In conclusion, low serum 1,25(OH)₂D₃ concentrations and high serum FGF23 concentrations in association with hypophosphataemia are reliable biomarkers for OOM (Fig. 1), which is a paraneoplastic disorder due to tumours that are often small and difficult to locate, as they may occur anywhere in the body. The majority of tumours causing OOM are PMTMCs, and the detection by immunohistochemistry of lymphatic endothelial cell markers such as LYVE-1 has been reported (10) to help to distinguish these tumours from other vascular tumours, as illustrated by our study (Fig. 3).

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