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Case Report

Elevated FGF23 in a patient with hypophosphatemic osteomalacia associated with neurofibromatosis type 1

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ABSTRACT

Context: The mechanism behind hypophosphatemia in the setting of neurofibromatosis type 1 (NF1) is not known. We describe a possible role of fibroblast growth factor-23 (FGF23) in the pathophysiology of hypophosphatemia in a patient with NF1.

Case description: A 34-year woman with NF1 presented with severe hypophosphatemia, osteomalacia, and elevated plasma FGF23. The patient had considerable improvement on replacement of oral phosphate. Two Ga68 DOTANOC PET-CT scans over a period of 2 years failed to detect any localized uptake. Immuno-staining for FGF23 was absent in the neural-derived tumour cells of the neurofibromas in the proband.

Conclusion: The patient with NF1 had elevated circulating FGF23. Tumour cells in the neurofibroma tissues did not stain for FGF23 on IHC. It is unlikely for neurofibromas to contribute to high circulating FGF23 levels in the proband.

Neurofibromatosis type 1 (NF1, OMIM 162200) is an autosomal dominant disorder with varied manifestations. It results from inactivating mutations in the neurofibromin gene, leading to activation of the *RAS* and its downstream signaling pathways [1]. Unlike skeletal abnormalities, hypophosphatemic osteomalacia is rarely encountered in NF1, with < 40 such cases reported till date [2–5]. The etiology of hypophosphatemia in patients with NF1 is currently unknown. Proximal renal tubular acidosis [4] and primary hyperparathyroidism [5] have been described as causes for hypophosphatemia in single cases. It has also been hypothesized that excessive production of fibroblast growth factor-23 (FGF23) from a neurofibroma may be responsible for chronic hypophosphatemia [3]. However, marginally elevated circulating FGF23 was reported only in a single patient of NF1 and the exact pathophysiology behind this has not been studied [3].

Recently, the activated *RAS* pathway has been hypothesized to play a key role in excessive production of FGF23 and hypophosphatemic osteomalacia in patients with epidermal and melanocytic nevi (cutaneous skeletal hypophosphatemia syndrome) [6,7]. Similarly, immunohistochemical staining for FGF23 and a point mutation in *KRAS* protooncogene has been demonstrated in a patient of metastatic adenocarcinoma of colon [8]. In a recent study involving a *Nf1* conditional knockout mouse model (*Nf1 cKO*), it was demonstrated that bone was the source of excessive FGF23 production as assessed by immunohistochemistry (IHC) and RTPCR [9]. The FGF23 production was specifically inhibited by an inhibitor of PI3 kinase (one of the downstream signaling pathways of activated RAS) [9]. Furthermore, an association between the activation of AKT pathway and FGF23 production was shown in a patient with PTEN-negative Cowden syndrome [10]. We describe a patient of NF1 with severe hypophosphatemia, osteomalacia and elevated plasma FGF23. We aimed to study the expression of FGF23 in the bone, neurofibroma and skin of the proband to determine the source of circulating FGF23.

Abbreviations: 25OHD, 25-hydroxy vitamin D; BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; FGF23, fibroblast growth factor-23; IHC, immunohistochemistry; NF1, neurofibromatosis type 1; PTH, parathyroid hormone; RAS, rat sarcoma; Tmp-GFR, tubular maximum reabsorption of phosphate-glomerular filtration rate

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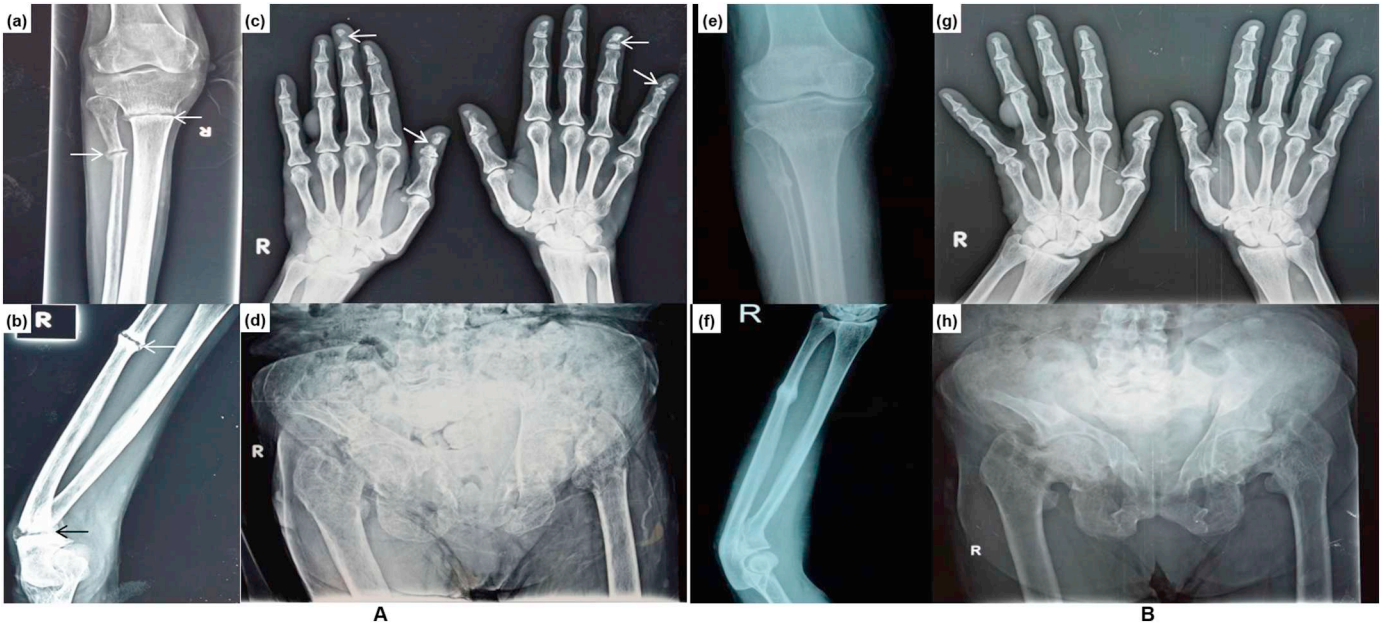

Fig. 1. Skeletal survey of the patient before (A) and after (B) therapy.
Skeletal survey at presentation showing generalized osteopenia, pseudo-fractures at (a) proximal end of right tibia and fibula, (b) mid-shaft and proximal end of right ulna, (c) multiple pseudo-fractures and subperiosteal bone resorption in both the hands, (d) fractures at both neck of femur and tri-radiate pelvis, (e–h) one and half years after treatment, there is evidence of healing of the above changes.
Arrows denote pseudo-fractures.

1. Case report

A 34-year female with widespread neurofibromas was diagnosed as NF1, as defined by NIH criteria [11]. She presented in 2013 with 10-years duration of low-back pain, generalized bony aches, height loss of 32 cm (current height 119 cm), and progressive bowing of all 4 extremities. She had increasing proximal myopathy and was bed-ridden 6 months prior to her presentation. The symptoms did not improve despite calcium and vitamin D supplementation. Her mother, sister, and both offspring also met the clinical criteria for NF1, but without skeletal manifestations. All other family members with NF1 had few neurofibromas (Supplement Fig. 1). Next-generation sequencing (Illumina HiSeq 4000 System, San Diego, CA) revealed a pathogenic heterozygous 4 bp deletion in the exon 16 of *NF1* gene in the proband (c.1756_1759del).

Skeletal survey revealed fractures at both neck of femur, multiple pseudo-fractures in the long bones and both hands, bowing of long bones, tri-radiate pelvis and kyphoscoliosis (Fig. 1a–d). She had severe osteoporosis (Z-score at lumbar spine −6.8 on DXA scan). The patient had low serum phosphorous [1.6 mg/dl (reference range 2.5–4.5 mg/dl)] and Tmp-GFR [1.1 (reference range 2.5–4.4)], elevated levels of serum alkaline phosphatase and intact PTH (Table 1), and normal levels of serum calcium and 25OHD. Serum 1,25-dihydroxyvitamin D was inappropriately normal [53 pg/ml (reference range 16–56 pg/ml)], despite low phosphorous and high PTH levels. Markers of bone formation and resorption were elevated. She had a normal plasma haemoglobin (14.1 g/dl) and haematocrit (44%). Plasma C-terminal FGF23 (Immutopics, San Clemente, CA; intra-assay and inter-assay CV < 10%) and serum intact FGF23 (Kainos, Tokyo; intra-assay and inter-assay CV < 12%), repeated on 2 separate occasions, were elevated [C-terminal FGF23; 247 and 423 RU/ml (reference range < 150 RU/ml), intact FGF23; 112 and 150 pg/ml (reference range 10–50 pg/ml)].

Proximal renal tubular acidosis was excluded by absence of renal glycosuria, aminoaciduria and metabolic acidosis. Ga68 DOTANOC PET-CT scan done on two occasions, 2 years apart, did not reveal any tumoral uptake. Autosomal dominant hypophosphatemic disorder was ruled out by sequencing the FGF23 gene. The patient was treated with oral phosphorous supplementation (1 g/d) and calcitriol (0.75 μg/d). Her symptoms improved considerably, and she is currently ambulatory without requiring any support and able to perform her daily activities. The radiological healing of osteomalacia was noted (Fig. 1e–f). Repeat DXA scan showed marked improvement in BMD (Z-score −2.8 at lumbar spine). The patient had no additional manifestations of NF1 till the last visit in January 2018. A sister, who met the clinical criteria for NF1, had normal biochemical parameters and FGF23 levels (plasma C-terminal FGF23 level 134 RU/ml; serum intact FGF23 level 29 pg/ml).

Table 1
Biochemical parameters of the proband with NF1.

Parameter	Report	Reference range
Serum total corrected calcium (mg/dl)	8.9	8.5–10.8
Serum phosphorous (mg/dl)	1.6	2.5–4.5
Tmp-GFR	1.1	2.5–4.4
Serum alkaline phosphatase (U/L)	566	35–150
Serum creatinine (mg/dl)	0.65	0.5–1.6
Serum 25OHD (ng/ml)[a]	30	> 20
Plasma intact PTH (pg/ml)	88	9–55
Serum 1,25 OHD3 (pg/ml)	53	16–56
Plasma C-terminal FGF23 (RU/ml)[b]	423, 247	< 150
Serum intact FGF23 (pg/ml)[b]	150, 112	10–50
Plasma C-terminal telopeptide (pg/ml)	1824	25–573
Plasma osteocalcin (ng/ml)	124	11–43
Plasma bone-specific alkaline phosphatase (U/L)	66	11.6–29.6
Serum procollagen type I amino-terminal propeptide (PINP, ng/ml)	183	19–83

[a] Patient on vitamin D replacement.
[b] FGF23 measured on two separate occasions.

2. Materials and methods

The written informed consent of the subjects was obtained. The study methods were approved by the institutional ethics committee. Biopsy specimens of the proband's pelvic bone, two neurofibromas and skin were obtained.

2.1. Immuno-histochemical staining for FGF23

Formalin-fixed paraffin-embedded tissue sections from neurofibromas and skin, from the proband and her sister (with NF1 but normal serum phosphorous) were examined for FGF23 expression. Unfortunately, the bone sections available were inadequate for IHC, despite numerous attempts, because of markedly porous and fragile bone. Specimens from 5 patients with sporadic neurofibroma and skin from two healthy adults were also examined for FGF23 IHC. Tumour specimens from two patients with proven oncogenic osteomalacia acted as positive controls. The sections were separately stained with two different anti-FGF23 antibodies (anti-FGF23, Adipogen, San Diego, CA; at a dilution of 1:150, and FGF-23 Ab, C-term 225-244, Immutopics, San Clemente, CA; at a dilution of 1:100). The sections were incubated with primary antibody at room temperature for 24 h (Adipogen) and 4 °C overnight (Immutopics) respectively. Di-amino-benzidine (UltraVision Quanto HRP DAB, Thermo Fisher Scientific, Fremont, CA) was used for detection of the bound antibody. Cytoplasmic granular expression was interpreted as positive staining. Mast cells from specimens of chronic cystitis, mastocytosis and nasal mucosa (patient with chronic rhinitis) were also examined for FGF23 expression. Specificity of staining was studied by using antibody isotype-negative control (mouse IgG1*k* isotype, Adipogen, San Diego, CA) and peptide blocking (FGF23, Adipogen, San Diego, CA). Experiments were also conducted after omitting the primary antibody.

3. Results

3.1. Immunohistochemistry for FGF23 expression

The tumour cells in the neurofibromas were negative for FGF23 staining as assessed by IHC using both the primary antibodies (Fig. 2d and h). However, infiltrating mast cells demonstrated strong positive staining for FGF23 in the neurofibromas from the proband and sister (Fig. 2d–e) using the first anti-FGF23 antibody (Adipogen). The mast cells infiltrating sporadic neurofibromas and unrelated disorders were also stained for FGF23 using this antibody (Supplement Fig.2). On use of antibody isotype-negative control and also by peptide blocking, the neurofibroma of proband, sister, sporadic neurofibroma and unrelated disorders did not show immuno-staining in the mast cells (Fig. 2g and Supplement Fig. 2). IHC using the second primary antibody (Immutopics) did not stain the mast cells in any of the above-mentioned specimens (Fig. 2h). These data suggest that staining of mast cells with the first antibody is most likely due to cross-reactivity with related products in the mast cells.

4. Discussion

The coexistence of skeletal dysplasia, neurofibroma, hypophosphatemia and unequivocally elevated plasma FGF23 in a patient of NF1 has not been previously reported. Though hypophosphatemia has been described in several patients with NF1 [2–5], FGF23 has previously been measured in only three patients, one of whom had a marginally elevated level of plasma FGF23 (151 RU/ml, reference range < 150 RU/ml) [3]. Resection of the neurofibroma in a patient with increased uptake in a large neurofibroma (as determined by ^{18}FDG PET-CT scan) did not improve hypophosphatemia [3].

In the present report, FGF23 staining was absent in the neural-derived tumour cells in the neurofibroma. The mast cells demonstrated FGF23 staining in the neurofibromas of the proband, her sister (with NF1 and with normal biochemistry), sporadic neurofibromas and unrelated disorders using first primary antibody (from Adipogen). However, FGF23 staining in the mast cells was absent in all above-mentioned specimens when the sections were stained with a different primary antibody (Immutopics). Hence, it can be presumed that the FGF23 staining with the first primary antibody might be a cross reactivity with some other related protein in the mast cells. Taken together, IHC data suggests neurofibromas are unlikely source of excess circulatory FGF23 levels and the bone is probably the primary source of circulatory FGF23 in the proband. Supporting this hypothesis, a recent study has shown that bone is the source of FGF23 in a *Nf1 cKO* mouse model [9].

In patients with NF1, inactivating mutations in the neurofibromin gene result in activation of *RAS* and its downstream signaling pathways i.e. PI3K/Akt/mTOR and MAPK/ERK pathways [12]. Overexpression of FGF23 and mutation in *KRAS* protooncogene in the same tissue has been described in a case of metastatic adenocarcinoma of the colon [8]. Activation of *RAS* and FGF23 mediated hypophosphatemia has also been reported in epidermal nevus syndrome [6,7]. Bone was thought to be the source of excess FGF23 in these studies, though bone sections were not analyzed for FGF23 expression [6,7]. A study involving *Nf1 cKO* mouse showed that PI3K pathway activation might be responsible for increased FGF23 production [9]. The upregulation of FGF23 was specifically inhibited by Ly294002, a PI3K inhibitor. Taken together,

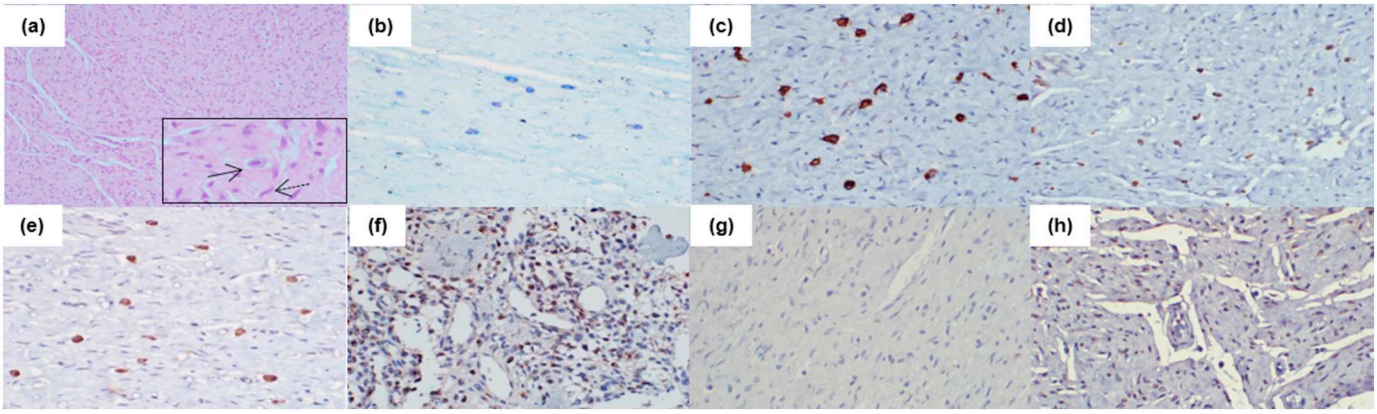

Fig. 2. Expression of FGF23.
(a) Histopathology of neurofibroma stained with hematoxylin-eosin (10×). Higher magnification (80×, inlet panel a) shows a mast cell (solid arrow) and tumour cells (broken arrow). Positive staining with (b) Toluidine blue and (c) CD117 in the proband's neurofibroma confirmed the presence of mast cells. Immunohistochemical staining with FGF23 antibody (Adipogen) of the (d) proband's and (e) sister's neurofibroma showing mast cells staining positive for FGF23 (brown colour, cytoplasmic staining), (f) staining of sections from a proven oncogenic osteomalacia (positive control), (g) IHC done after with peptide blocking resulting in no staining of mast cells for FGF23, (h) IHC of proband's neurofibroma with a different primary antibody (FGF23 Ab, C-terminal 225-244, Immutopics) did not stain the mast cells for FGF23, (images b-h are in 40× magnification). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

activation of *RAS* and its downstream signaling pathways in NF1 may result in excess and autonomous production of FGF23 from osteocytes and *RAS* may be the missing link in some of the FGF23 mediated hypophosphatemia.

Our study had certain limitations. The available bone specimens from the proband were inadequate for IHC. Similarly, the bone tissue was inadequate to detect a "second hit (i.e. mutations in *PTEN*)" in the patient which might have resulted in a more severe phenotype compared to other family members with NF1. However, sequencing of the *PTEN* gene in the genomic DNA did not reveal a mutation.

To conclude, we report a patient with NF1 with hypophosphatemic osteomalacia and elevated circulating FGF23. Correction of hypophosphatemia resulted in marked clinical improvement. The neurofibromas were unlikely to be the source of FGF23 in the patient. Though we were unable to demonstrate directly, bone is the most likely source of high circulatory FGF23. It is feasible that activation of the *RAS* mediated pathways in NF1 may play a role in overexpression of FGF23 from bone. Further studies are necessary to elucidate the role of this pathway in patients with NF1 and hypophosphatemia.

Authorship

EB, SKS were responsible for the conception and design of the study, acquisition of data, and analysis and interpretation of data. PK, NB, VK, RT, VA, NA, GZ, NI and LP were responsible for conduction of experiments, data analysis and interpretation. SKS, PK, EB, VA and RT were responsible for drafting the article and revising it critically for important intellectual content. All authors approved the final version of the manuscript.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.115055.

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