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RESEARCH LETTER

Evaluation of bone markers in hypophosphatemic rickets/osteomalacia

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Abstract N-terminal propeptide of type I procollagen (PINP) is a marker of newly formed type I collagen. However, its role in hypophosphatemic rickets/osteomalacia has not yet been established. Metabolic bone markers were examined in patients with oncogenic osteomalacia (OOM) and X-linked hypophosphatemic rickets (XLH), and in healthy controls. OOM and XLH patients were found to have hypophosphatemia secondary to elevated levels of serum fibroblast growth factor 23 (FGF-23). OOM patients had reduced levels of 1,25-dihydroxy vitamin D (1,25D) compared with XLH patients and healthy controls, despite attenuation of the reduction in these levels in the XLH patients secondary to active vitamin D supplementation. In contrast to patients with XLH, OOM patients showed a significant increase in serum PINP, which is suggestive of accelerated bone matrix formation. Bone alkaline phosphatase (BAP) and the BAP/PINP ratio were also increased in OOM but not in XLH patients, suggesting the presence of a disturbance in bone mineralization in OOM. Long-term supplementation of active form vitamin D and inorganic phosphate (IP) may have attenuated the defect in bone mineralization in the XLH patients, resulting in the normalization of PINP, BAP, and the BAP/PINP ratio. The present results suggest that, as with BAP, PINP is an appropriate metabolic bone marker in the assessment of hypophosphatemic rickets/osteomalacia.

Keywords PINP · Oncogenic osteomalacia · Tumor-induced osteomalacia · X-linked hypophosphatemic rickets

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Introduction

Circulating FGF-23, the phosphaturic hormone, is progressively increased to compensate for persistent phosphate retention in patients with chronic kidney disease [1]. Homeostases of Ca and phosphate involve feedback loops between Ca; phosphate; 1,25D; PTH; and FGF-23 [2]. An excess of FGF-23 is a major factor in the development of hypophosphatemic rickets/osteomalacia, including OOM and XLH [3].

Methods

This study examined seven OOM patients, nine XLH patients, and 23 healthy controls. Blood samples were obtained from all the participants after an overnight fast. Serum parameters were measured as described previously [1]. Statistical analyses were performed by Kruskal–Wallis test with Bonferroni correction for multiple testing of each group.

Results

All the participants had normal kidney function (Table 1). The significant reductions in serum phosphate and in tubular maximum phosphate reabsorption per glomerular

Table 1 Clinical characteristics of the study participants

	Normal range	Control	XLH	OOM
N (M/F)		23(6/17)	9(1/8)	7(5/2)
Age (years)		46.9 ± 2.2	40.6 ± 4.6	55.0 ± 3.7
Estimated GFR (ml/min/1.73 m^2)		82.2 ± 3.0	95.9 ± 9.3	86.5 ± 4.4
Corrected Ca (mg/dl)	8.2–10.2	9.60 ± 0.068	9.84 ± 0.119	9.39 ± 0.040[b]
IP (mg/dl)	2.8–4.3	3.57 ± 0.091	1.99 ± 0.068[a]	1.73 ± 0.097[a]
Whole PTH (ng/l)	9.0–39.0	26.6 ± 2.5	25.4 ± 3.8	36.4 ± 7.3
1,25D (pg/ml)	20.0–60.0	52.7 ± 3.8	47.3 ± 11.4	15.6 ± 3.0[a,b]
Tmp/GFR(mmol/l)	0.80–1.35	1.06 ± 0.031	0.52 ± 0.018[a]	0.41 ± 0.037[a]
FGF-23 (pg/ml)	14.7–40.5	28.8 ± 3.7	125.8 ± 36.0[a]	165.5 ± 60.2[a]
BAP (U/l)	9.5–35.4	24.1 ± 2.7	37.6 ± 7.7	201.2 ± 39.1[a,b]
Osteocalcin (ng/ml)	3.1–12.7	6.40 ± 0.56	7.61 ± 1.10	9.60 ± 1.09[a]
NTX (nmol/l)	9.5–17.7	16.7 ± 1.0	21.7 ± 2.1	22.2 ± 3.2

eGFR the estimated GFR calculated using the abbreviated modification of diet in renal disease (MDRD) study equation, modified with the Japanese coefficient; *Ca* calcium. Serum Ca levels were expressed as corrected Ca after adjustment for serum albumin. *IP* Inorganic phosphate, *PTH* parathyroid hormone, *1,25D* 1,25-dihydroxycholecalciferol, *TmP/GFR* tubular maximum phosphate reabsorption per glomerular filtration rate. TmP/GFR was calculated using data from a second-morning urine sample. *FGF-23* fibroblast growth factor 23, *BAP* bone alkaline phosphatase, *NTX* N-telopeptide of collagen type I

Results were denoted as mean ± standard deviation (SD). [a] $P < 0.0167$ verses healthy controls, [b] $P < 0.0167$ verses XLH after Bonferroni corrections

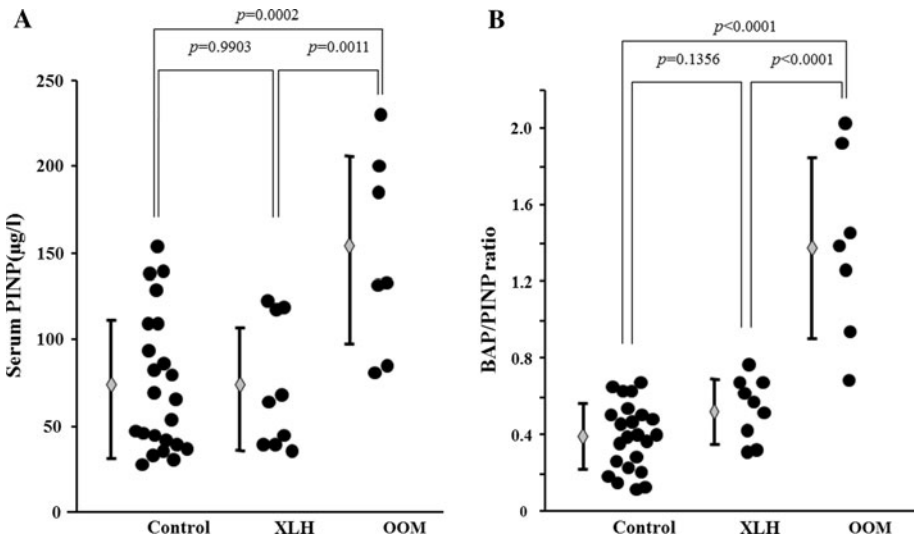

Fig. 1 **a** Serum PINP levels. OOM patients showed a significant increase compared with both the healthy controls ($P = 0.0002$) and the XLH patients ($P = 0.0011$) after Bonferroni corrections. **b** Serum BAP/PINP ratio. OOM patients showed a significant increase compared with both the healthy controls ($P < 0.0001$) and the XLH patients ($P < 0.0001$) after Bonferroni corrections. Values represent the mean ± SD

filtration rate (TmP/GFR), as well as the significant increase in serum FGF-23 level are consistent with the diagnosis of OOM and XLH.

A significant increase in serum PINP, BAP and osteocalcin were observed in the OOM patients (Fig. 1a; Table 1). No significant difference in serum N-telopeptide of collagen type I (NTX) concentration was observed between the three study groups. A significant increase in the BAP/PINP ratio was observed in the OOM patients (Fig. 1b). However, no significant difference in osteocalcin/PINP ratio was observed between the three study groups (data not shown).

Discussion

In the OOM patients, serum BAP levels were dramatically elevated, whereas only a mild significant increase in serum osteocalcin was observed (Table 1). Patients with rickets/osteomalacia, secondary to hypophosphatemia, vitamin D deficiency, gastrectomy, or gluten enteropathy display elevated levels of serum BAP and total alkaline phosphatase [4–6]. Serum osteocalcin is normal [5, 6] or mildly elevated [4] in rickets/osteomalacia.

A previous study reported a mild increase in PINP in three out of four patients with osteomalacia and a mild decrease in

PINP in one patient with XLH [6]. However, the sample size was too small to determine the role of PINP in hypophosphatemic diseases. In this study, the OOM patients showed a significant increase in PINP compared with XLH patients and healthy controls (Fig. 1a), suggesting that bone extracellular matrix production is enhanced in OOM.

Interestingly, the XLH patients showed no significant increase in BAP, osteocalcin, or PINP compared with the healthy controls (Table 1). Administration of supraphysiological amounts of active vitamin D has been shown to reverse histomorphometrical abnormalities [7] and to normalize bone density [8] in XLH. For the diagnostic utility to distinguish between XLH and OOM, BAP is superior to PINP (Table 1; Fig. 1a).

In the development of bone cells, sequential expression of osteoblast-specific genes occurs during the proliferation period before mineralization. The proliferation period is followed by the biosynthesis of the type I collagen bone extracellular matrix [9], indicating that PINP is an early stage marker of bone formation. After completion of the proliferation period, the peak level of ALP occurs, indicating the matrix maturation period. Subsequently, osteocalcin reaches its peak level, and calcium is then deposited in the matrix during the mineralization period. In the OOM patients, the BAP/PINP ratio was significantly increased (Fig. 1b), although no increase in the osteocalcin/PINP ratio was observed (data not shown), suggesting that OOM involves a mineralization defect. Hypophosphatemic transgenic mice that overexpress *FGF23* in osteoblasts display the reduced bone mineral density that is characteristic of rickets/osteomalacia [10]. Increased mRNA expression of ALP but not of osteocalcin has been demonstrated in the long bones of these transgenic mice. Taken together, these findings suggest that an increased BAP/PINP ratio could indicate a disturbance in bone mineralization, and that this mineralization defect is specific to OOM. Long-term administration of the active form of vitamin D and IP to patients with OOM would be necessary to confirm this hypothesis.

No significant difference in serum NTX suggested that the bone resorption is not a main cause for the bone abnormalities observed in these disorders.

In conclusion, as with BAP, PINP is an appropriate metabolic bone marker in the assessment of hypophosphatemic rickets/osteomalacia. The observed increase in the BAP/PINP ratio in OOM patients suggests the presence of a disturbance in bone mineralization. No increase in the osteocalcin/PINP ratio was observed in OOM patients, suggesting that OOM involves a mineralization defect. The long-term supplementation of the active form of vitamin D and IP in the XLH patients may have attenuated the bone mineralization disturbance, resulting in the normalization of PINP, BAP, and the BAP/PINP ratio.

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Ethical standards All participants provided written informed consent before inclusion. This study was approved by the ethics committee of the Osaka City University Graduate School of Medicine and was conducted in accordance with the principles of the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Y. Imanishi, M. Inaba, K. Nakatsuka, K. Nagasue, S. Okuno, A. Yoshihara, M. Miura, A. Miyauchi, K. Kobayashi, T. Miki, T. Shoji, E. Ishimura, Y. Nishizawa, FGF-23 in patients with end-stage renal disease on hemodialysis. Kidney Int. **65**(5), 1943–1946 (2004)
2. Y. Imanishi, M. Inaba, T. Kawata, Y. Nishizawa, Animal models of hyperfunctioning parathyroid diseases for drug development. Expert Opin. Drug Discov. **4**(7), 727–740 (2009)
3. K.B. Jonsson, R. Zahradnik, T. Larsson, K.E. White, T. Sugimoto, Y. Imanishi, T. Yamamoto, G. Hampson, H. Koshiyama, O. Ljunggren, K. Oba, I.M. Yang, A. Miyauchi, M.J. Econs, J. Lavigne, H. Juppner, Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. N. Engl. J. Med. **348**(17), 1656–1663 (2003)
4. B. Demiaux, M.E. Arlot, M.C. Chapuy, P.J. Meunier, P.D. Delmas, Serum osteocalcin is increased in patients with osteomalacia: correlations with biochemical and histomorphometric findings. J. Clin. Endocrinol. Metab. **74**(5), 1146–1151 (1992)
5. E.D. Daniels, J.M. Pettifor, G.P. Moodley, Serum osteocalcin has limited usefulness as a diagnostic marker for rickets. Eur. J. Pediatr. **159**(10), 730–733 (2000)
6. I. Ros, L. Alvarez, N. Guanabens, P. Peris, A. Monegal, I. Vazquez, D. Cerda, A.M. Ballesta, J. Munoz-Gomez, Hypophosphatemic osteomalacia: a report of five cases and evaluation of bone markers. J. Bone Miner. Metab. **23**(3), 266–269 (2005)
7. R.M. Harrell, K.W. Lyles, J.M. Harrelson, N.E. Friedman, M.K. Drezner, Healing of bone disease in X-linked hypophosphatemic rickets/osteomalacia. Induction and maintenance with phosphorus and calcitriol. J. Clin. Invest. **75**(6), 1858–1868 (1985)
8. I.R. Reid, W.A. Murphy, D.C. Hardy, S.L. Teitelbaum, M.A. Bergfeld, M.P. Whyte, X-linked hypophosphatemia: skeletal mass in adults assessed by histomorphometry, computed tomography, and absorptiometry. Am. J. Med. **90**(1), 63–69 (1991)
9. G.S. Stein, J.B. Lian, Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. Endocr. Rev. **14**(4), 424–442 (1993)
10. K. Hollberg, R. Marsell, M. Norgard, T. Larsson, K.B. Jonsson, G. Andersson, Osteoclast polarization is not required for degradation of bone matrix in rachitic FGF23 transgenic mice. Bone **42**(6), 1111–1121 (2008)