Table 2 FGF23-related hypophosphatemic rickets and osteomalacia

X-linked dominant hypophosphatemic rickets/osteomalacia (XLH): mutations in the *PHEX* gene
Autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR): mutations in *FGF23* gene
Autosomal recessive hypophosphatemic rickets/osteomalacia 1 (ARHR1): mutations in the *DMP1* gene
Autosomal recessive hypophosphatemic rickets/osteomalacia 2 (ARHR2): mutations in the *ENPP1* gene
Hypophosphatemic disease with dental anomalies and ectopic calcification: mutations in the *FAM20C* gene
McCune–Albright syndrome/fibrous dysplasia
Linear sebaceous nevus syndrome
Tumor-induced rickets/osteomalacia
Hypophosphatemic rickets/osteomalacia caused by saccharated ferric oxide or iron polymaltose

DMP1 dentin matrix acidic phosphoprotein 1, *ENPP1* ectonucleotide pyrophosphatase/phosphodiesterase 1, *FAM20C* family with sequence similarity 20, member C, *FGF23* fibroblast growth factor 23, *PHEX* phosphate-regulating endopeptidase homolog, X-linked

the development of hypophosphatemia in these patients. Vitamin D deficiency is diagnosed by low serum 25(OH) D levels. Some patients with vitamin D deficiency also present with secondary hyperparathyroidism. Because parathyroid hormone stimulates conversion of 25(OH)D to $1,25(OH)_2D$, patients with vitamin D deficiency can present with various levels of $1,25(OH)_2D$. Therefore, vitamin D deficiency cannot be diagnosed by $1,25(OH)_2D$ levels.

Gastrectomy or enterectomy can cause osteomalacia [8]. It is possible that after these surgical procedures, osteomalacia is not properly diagnosed and treated in patients. The causes of osteomalacia in these patients may be multifactorial, including vitamin D deficiency and impaired mineral absorption. Although liver cirrhosis or chronic liver disease was described as one of the causes of osteomalacia, osteoporosis seems to be much commoner than osteomalacia in these patients [9].

There are many causes of renal phosphate wasting. About 80–90 % of phosphate filtered from glomeruli is absorbed in renal proximal tubules. Type 2a and 2c sodium–phosphate cotransporters mediate physiological phosphate reabsorption in proximal tubules [10]. Renal phosphate wasting can be observed in patients with Fanconi syndrome and some form of renal tubular acidosis. In addition, hereditary hypophosphatemic rickets with hypercalciuria is caused by mutations in *SLC34A3*, which encodes type 2c sodium–phosphate cotransporter [11, 12]. Dent disease is characterized by low molecular weight proteinuria, hypophosphatemia, and nephrolithiasis. This disease is caused by mutations in *CLC5*, which encodes a chloride channel [13]. In addition to these intrinsic renal tubular defect, excessive actions of FGF23 cause hypophosphatemia with enhanced renal phosphate excretion. FGF23 suppresses renal tubular phosphate reabsorption by reducing the expression of type 2a and 2c sodium–phosphate cotransporters [14]. In addition, FGF23 decreases serum $1,25(OH)_2D$ levels by altering the expression levels of vitamin D-metabolizing enzymes [14]. Therefore, FGF23 reduces serum phosphate level by suppressing renal phosphate reabsorption and inhibiting intestinal phosphate absorption through lowering $1,25(OH)_2D$ levels.

After the identification of FGF23, it became clear that patients with some kinds of hypophosphatemic rickets and osteomalacia, such as X-linked hypophosphatemic rickets (XLH) and tumor-induced osteomalacia (TIO), a rare paraneoplastic syndrome, show high circulatory levels of FGF23 [15, 16]. In contrast, FGF23 levels in patients with rickets or osteomalacia from other causes, including vitamin D deficiency, were rather low [5]. Together with the biological activities of FGF23 mentioned above, these results indicated that FGF23 is the humoral factor that causes hypophosphatemia in patients with several diseases, including XLH and TIO. Since then, other hypophosphatemic diseases have also been shown to be associated with high FGF23 levels [17] (Table 2). It is believed that FGF23 production in bone is enhanced in most patients with these FGF23-related hypophosphatemic diseases except for TIO. However, the precise mechanisms of overproduction of FGF23 and the actions of the gene products responsible for genetic FGF23-related hypophosphatemic diseases are largely unknown.

Diagnostic criteria

Because the clinical presentations of rickets and osteomalacia are quite different, it is not practical to prepare one kind of diagnostic criteria for both of these diseases. Therefore, we propose the following diagnostic criteria (Table 3). Rickets is basically diagnosed by the presence of rachitic changes of bones such as cupping and fraying of the metaphysis, and widening of the epiphyseal plate observed by X-ray. In addition, high alkaline phosphatase level is characteristic of diseases with impaired mineralization. We believe that these two laboratory findings are essential to make a diagnosis of rickets or suspect the presence of this

Table 3 Diagnostic criteria for rickets and osteomalacia

Rickets	Osteomalacia
(a) Rachitic changes by X-ray (cupping and fraying of the metaphysis, widening of the epiphyseal plate)	(a) Hypophosphatemia or hyocalcemia[a]
(b) High alkaline phosphatase level	(b) High bone alkaline phosphatase level
(c) Hypophosphatemia or hypocalcemia[a]	(c) Clinical symptoms: muscle weakness or bone pain
(d) Clinical signs: bone deformities such as genu varum and genu valgum, abnormal spinal curvature, craniotabes, open fontanels, rachitic rosary, joint swelling	(d) Low BMD: less than 80 % of YAM
Definite rickets: patients who have (a)–(d)	(e) Abnormal imaging findings: multiple uptake by bone scintigraphy or Looser's zone by X-ray
Possible rickets: patients who have (a), (b), and 1 of (c) or (d)	Definite osteomalacia: patients who have (a)–(e)
	Possible osteomalacia: patients who have (a), (b), and 2 of (c)–(e)

BMD bone mineral density, *YAM* young adult mean

[a] Does not apply to patients with inhibitors of mineralization

Table 4 Diseases that need to be discriminated form rickets and osteomalacia

Symptoms	Diseases
Low BMD	Osteoporosis, renal osteodystrophy, primary hyperparathyroidism
Bone deformity	Skeletal dysplasia
Bone pain	Polymyalgia rheumatica, ankylosing spondylitis
Muscle weakness	Neuromuscular diseases
Multiple uptake by bone scintigraph	Multiple metastases
Rachitic change	Hypophosphatasia
High bone alkaline phosphatase level	Primary hyperparathyroidism, renal osteodystrophy, multiple metastases

BMD bone mineral density

disease. In addition, patients with rickets usually show hypophosphatemia or hypocalcemia, and some clinical signs. However, it is possible that patients show no clinical signs especially when the diagnosis of rickets is established in the early phase of the disease. This can happen in family members of affected patients with rickets from genetic causes. Therefore, we propose that patients with all four of these findings (a–d for rickets in Table 3) are regarded as definitely having rickets, and patients who lack either hypophosphatemia or hypocalcemia, or clinical signs (c or d for rickets in Table 3) are regarding as possibly having rickets.

In contrast to rickets, there is no single laboratory test which strongly suggests the presence of osteomalacia. However, patients with osteomalacia usually show either hypophosphatemia or hypocalcemia. High alkaline phosphatase level is also seen in patients with osteomalacia. Although almost all alkaline phosphatase activity in blood derives from bone in children, there is a significant contribution from the liver and other organs in adults. We believe that either hypophosphatemia or hypocalcemia, and high bone alkaline phosphatase level are essential to diagnose osteomalacia or suspect this disease. In addition, patients with osteomalacia may show some clinical symptoms, low bone mineral density (BMD), multiple uptake by bone scintigraphy, or Looser's zone. However, patients may not show these findings when osteomalacia is suspected early in the course of the disease as in the case of rickets. Therefore, we propose that patients with all five of these findings (a–e for osteomalacia in Table 3) are regarded as definitely having osteomalacia, and patients who lack clinical symptoms, low BMD, or abnormal imaging findings (c–e for osteomalacia in Table 3) are regarding as possibly having osteomalacia.

Drugs that inhibit mineralization can induce rickets or osteomalacia without changing serum calcium or phosphate levels. In addition, several diseases need to be discriminated from rickets and osteomalacia (Table 4). The diseases listed in Table 4 can mimic some features of rickets or osteomalacia. On the other hand, most of these diseases cannot be diagnosed as rickets or osteomalacia with use of the diagnostic criteria given in Table 3. The exceptions are multiple metastases, primary hyperparathyroidism, and renal osteodystrophy. Osteoblastic bone metastases can cause either hypocalcemia or hypophosphatemia, high bone alkaline phosphatase level, bone

pain, and multiple uptake by bone scintigraphy. This possibility needs to be considered before the diagnosis of osteomalacia is finalized. Patients with primary hyperparathyroidism may also show hypophosphatemia, high bone alkaline phosphatase level, bone pain, low BMD, and multiple uptake by bone scintigraphy. However, hypercalcemia, which is rare in patients with osteomalacia, is usually present in patients with primary hyperparathyroidism. Patients with renal osteodystrophy may show hypocalcemia, high bone alkaline phosphatase level, bone pain, low BMD, and multiple uptake by bone scintigraphy. On the other hand, hyperphosphatemia rather than hypophosphatemia is usually observed in patients with renal osteodystrophy.

Hypophosphatasia is caused by mutations in *ALPL* (also known as *TNSALP* and *TNAP*), which encodes tissue-nonspecific alkaline phosphatase [18]. This disease is also characterized by impaired mineralization because alkaline phosphatase converts pyrophosphate, with potent inhibitory effects on mineralization, to phosphate. In this sense, hypophosphatasia can be regarded as one cause of rickets. However, patients with hypophosphatasia show low alkaline phosphatase levels, in contrast to those with rickets from other causes. Therefore, hypophosphatasia was treated as a disease that should be discriminated from rickets rather than a cause of rickets to avoid confusion in this proposal.

Differential diagnosis of causes of rickets and osteomalacia

After rickets or osteomalacia has been diagnosed, it is necessary to find the exact cause of these diseases. Table 5 summarizes typical biochemical changes observed in patients with rickets and osteomalacia from various causes. Vitamin D deficiency is defined by low 25(OH)D levels as mentioned before. Therefore, patients with low 25(OH)D levels are theoretically considered to have vitamin D deficient rickets or osteomalacia. However, vitamin D deficiency or insufficiency is quite common even in the general population [19], and it is possible that low 25(OH)D levels can be observed in patients with rickets or osteomalacia from other causes. Investigation of 25(OH)D levels in patients with vitamin D deficient rickets and XLH indicated that there was overlap of serum 25(OH)D levels in these patients. In contrast, FGF23 levels completely discriminated patients with vitamin D deficient rickets and XLH [20]. From these results, we propose a flowchart for differentiating various causes of rickets and osteomalacia (Fig. 1). In patients with hypophosphatemic rickets or osteomalacia, high FGF23 levels indicate FGF23-related hypophosphatemic diseases (Table 2). Vitamin D deficient rickets or osteomalacia is diagnosed after FGF23-related hypophosphatemic diseases, phosphate depletion, and other causes of renal tubular phosphate wasting have been ruled out. Vitamin D-dependent rickets type 1 and type 2 can be differentiated by $1,25(OH)_2D$ levels. Hereditary hypophosphatemic rickets with hypercalciuria is also characterized by high $1,25(OH)_2D$ levels. In normophosphatemic patients, use of drugs that inhibit mineralization and vitamin D deficiency should be considered. Patients with vitamin D deficiency may not show frank hypophosphatemia or hypocalcemia. It is possible that serum phosphate and calcium levels remain in the low normal range in these patients.

Discussion

Rickets and osteomalacia are not common diseases. However, quality of life of the affected patients can be severely compromised. For example, some untreated patients with TIO can become completely bedridden because of severe

Table 5 Typical biochemical findings in patients with rickets or osteomalacia from various causes

	Serum calcium	Serum phosphate	TmP/GFR	BAP	$1,25(OH)_2D$	25(OH)D	FGF23
FGF23-related hypophosphatemic disease	↓→	↓	↓	↑	↓→	→	⇧
Phosphate depletion	→	↓	↑	↑	→↑	→	↓→
Fanconi syndrome	→	↓	↓	↑	↓→	→	↓→
Vitamin D-dependent rickets type 1	↓	↓	↓	↑	↓	→	↓→
Vitamin D-dependent rickets type 2	↓	↓	↓	↑	↑	→	↓→
HHRH	→	↓	↓	↑	↑	→	↓→
Vitamin D deficiency	↓→	↓→	↓→	↑	→↑↓	⇩	↓→
Drugs that inhibit mineralization	→	→	→	↑	→	→	→

These thick down and up arrows indicate the specific laboratory tests for each category

BAP bone alkaline phosphatase, *25(OH)D* 25-hydroxyvitamin D, *$1,25(OH)_2D$* 1,25-dihydroxyvitamin D, *FGF23* fibroblast growth factor 23, *HHRH* hereditary hypophosphatemic rickets with hypercalciuria, *TmP/GFR* tubular maximum transport of phosphate per unit glomerular filtration rate

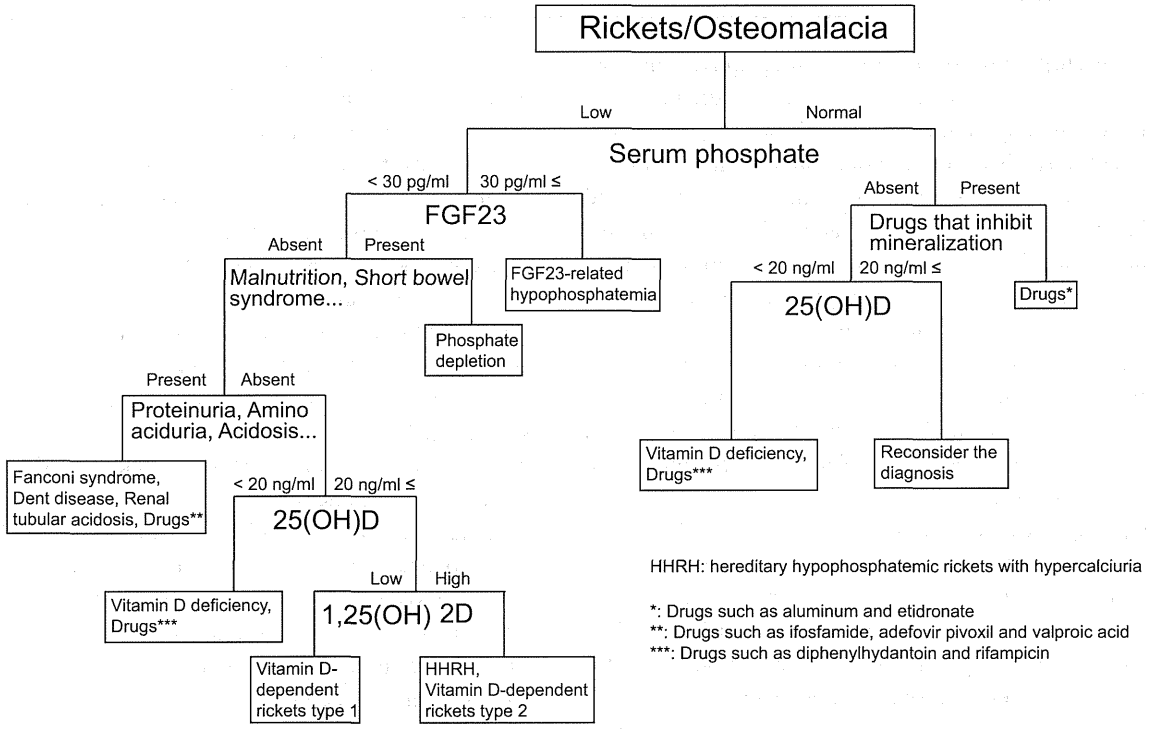

Fig. 1 Flowchart for the differential diagnosis of causes of rickets and osteomalacia. The causes of rickets and osteomalacia can be identified by several clinical findings and laboratory tests. *25(OH)D* 25-hydroxyvitamin D, *1,25(OH)$_2$D* 1,25-dihydroxyvitamin D, *FGF23* fibroblast growth factor 23

muscle weakness and bone pain. Short stature and bone deformities are big problems for patients with rickets. However, patients with rickets or osteomalacia can be completely cured or at least respond to treatment when rickets or osteomalacia is properly diagnosed and treated according to the specific causes. Therefore, we have listed causes of rickets and osteomalacia, and proposed diagnostic criteria and a flowchart for the differential diagnosis of various causes of these diseases.

There are several limitations to the proposed diagnostic criteria. These criteria were not created by retrospective review of clinical presentations of a large number of patients, but were proposed by several researchers and clinicians on the basis of their experiences. Therefore, the validity of these criteria and the flowchart needs to be examined in further studies. However, without any diagnostic criteria, it would be difficult for general medical professionals to correctly diagnose not-so-common illnesses. We hope that this proposal will become momentum for propagation of proper knowledge of rickets and osteomalacia, and for accumulation of more clinical data for revision of the criteria. In addition, there was a discussion about hypophosphatasia among us. This disease can be considered to be one cause of rickets. However, if hypophosphatasia is included in the causes of rickets, high alkaline phosphatase level cannot be used as one of the criteria for the diagnosis of rickets, and the flowchart for the differential diagnosis of various causes needs to be more complex. Because hypophosphatasia is rarer than other causes of rickets, such as vitamin D deficiency and XLH, and the easily usable diagnostic criteria for rickets and osteomalacia were planned, hypophosphatasia was not included as a cause of rickets in this proposal. Finally, measurements of FGF23 and 25(OH)D levels are not covered by medical insurance in Japan and are not included in routine laboratory tests. In contrast, these measurements are done by several commercial and research laboratories. We hope that this proposal will contribute to some extent to the future coverage of these measurements by medical insurance in Japan.

In summary, we have created diagnostic criteria and a flowchart for the differential diagnosis of various causes of rickets and osteomalacia. We hope that these criteria and the flowchart are clinically useful for the proper diagnosis and management of patients with rickets and osteomalacia.

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

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OPINION

Pathogenesis and diagnostic criteria for rickets and osteomalacia —Proposal by an expert panel supported by Ministry of Health, Labour and Welfare, Japan, The Japanese Society for Bone and Mineral Research and The Japan Endocrine Society

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Abstract. Rickets and osteomalacia are diseases characterized by impaired mineralization of bone matrix. Recent investigations revealed that the causes for rickets and osteomalacia are quite variable. While these diseases can severely impair the quality of life of the affected patients, rickets and osteomalacia can be completely cured or at least respond to treatment when properly diagnosed and treated according to the specific causes. On the other hand, there are no standard criteria to diagnose rickets or osteomalacia nationally and internationally. Therefore, we summarize the definition and pathogenesis of rickets and osteomalacia, and propose the diagnostic criteria and a flowchart for the differential diagnosis of various causes for these diseases. We hope that these criteria and flowchart are clinically useful for the proper diagnosis and management of patients with these diseases.

Key words: Vitamin D, Hypophosphatemia, Hyopcalcemia, FGF23

RICKETS and osteomalacia are diseases characterized by impaired mineralization of bone matrix [1, 2]. While rickets and osteomalacia are caused by the same etiologies, rickets develops before the closure of the growth plates. These diseases have been classified as metabolic bone diseases and endocrinologists have not considered these as endocrine diseases. Historically, nutritional vitamin D deficient rickets and osteomalacia were clinically very important [3]. Studies about this vitamin D deficient diseases led to the identification of 25-hydroxyvitain D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Since then, many causes of rickets and osteomalacia have been identified. Especially, recent studies established that fibroblast growth factor 23 (FGF23) is a phosphotropic hormone produced by bone and excessive actions of FGF23 cause several kinds of hypophosphatemic rickets and osteomalacia [4]. These results indicate that at least some kinds of hypophosphatemic rickets and osteomalacia are now considered to be endocrine diseases. Furthermore, it has been proposed that FGF23 measurement is useful for the differential diagnosis of hypophosphatemic diseases [5]. Therefore, it may be possible to newly classify causes of rickets and osteomalacia based on the pathophysiology utilizing these new findings.

There are no standard criteria to diagnose rickets or osteomalacia nationally and internationally. Since rickets and osteomalacia are not common lifestyle diseases, it may be difficult for general health professionals to properly diagnose and manage patients with these diseases. As impaired mineralization results in

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low bone mineral density measured by dual energy X-ray absorptiometry, osteomalacia must be discriminated from osteoporosis, a much commoner disease. Bone deformities observed in patients with rickets may lead to a wrong diagnosis like skeletal dysplasia. On the other hand, rickets and osteomalacia can severely impair the quality of life of the affected patients without proper diagnosis and management. From these backgrounds, it is clinically important to establish diagnostic criteria for rickets and osteomalacia that can be easily used by health professionals. In this review, we briefly summarize the definition and pathogenesis of rickets and osteomalacia, and propose the diagnostic criteria and investigation methods for the differential diagnosis of various causes for these diseases.

Definition and Clinical Presentations

Bone is a hard tissue created by deposition of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] on matrix proteins produced by osteoblasts. Because of the impairment of this mineralization of matrix proteins, unmineralized bone matrix (osteoid) increases in rickets and osteomalacia [1, 2]. Of these, rickets develops in children and osteomalacia is a disease in adulthood. Patients with rickets present several symptoms and signs including growth retardation, bone deformities such as genu valgum and varum, spinal curvature, craniotabes, open fontanels, rachitic rosary and joint swelling [1, 2]. Patients with osteomalacia may complain of bone pain and have muscle weakness, pigeon chest, spinal curvature and pseudofractures (Looser's zone) [1, 2].

Pathogenesis and Causes

Several drugs such as aluminum and etidronate directly inhibit mineralization of bone matrix proteins. In most other cases with rickets or osteomalacia, chronic hypophosphatemia, hypocalcemia, or both are present (Table 1). Hydroxyapatite crystals are formed in matrix vesicles produced by osteoblasts from calcium ion and phosphate. Hypophosphatemia or hypocalcemia is believed to impair mineralization by decreasing calcium x phosphate product.

Serum phosphate level is maintained by intestinal phosphate absorption, renal phosphate handling and phosphate movement between extracellular fluid and bone or intracellular fluid. Of these, chronic hypophosphatemia resulting in rickets and osteomalacia is usually caused by impaired intestinal phosphate absorption and/or renal phosphate wasting. Phosphate is abundant in food and phosphate deficiency does not occur in healthy subjects taking usual food. Phosphate deficiency may be observed in some patients suffering from malnutrition, malabsorption syndrome and so on.

Table 1 Causes of rickets/osteomalacia

Hypophosphatemia
Impaired actions of vitamin D metabolites
Vitamin D deficiency
Drugs (Diphenylhydantoin, Rifampicin, etc.)
Vitamin D-dependent rickets type 1[1)]
Vitamin D-dependent rickets type 2[2)]
Renal tubular dysfunction
Hereditary hypophosphatemic rickets with hypercalciuria[3)]
Fanconi syndrome
Dent disease[4)]
Renal tubular acidosis
Drugs (Ifosfamide, Adefovir pivoxil, Valproic acid, etc.)
FGF23-related hypophosphatemic rickets/osteomalacia (Table 2)
Phosphate depletion
Phosphate deficiency
Malabsorption…
Hypocalcemia
Some cases of vitamin D deficiency
Vitamin D-dependent rickets type 1[1)]
Vitamin D-dependent rickets type 2[2)]
Impaired mineralization from other causes
Drugs (Aluminum, Etidronate, etc.)

[1)] Mutations in *CYP27B1* gene, autosomal recessive
[2)] Mutations in *VDR* gene, autosomal recessive
[3)] Mutations in *SLC34A3* gene, autosomal recessive
[4)] Mutations in *CLCN5* gene, X-linked recessive

$1,25(OH)_2D$ enhances intestinal phosphate and calcium absorption, and impaired actions of vitamin D metabolites can result in hypophosphatemia and/or hypocalcemia. Drugs such as diphenylhydantoin and rifampicin may cause impaired actions of vitamin D metabolites by altering vitamin D metabolism. Vitamin D-dependent rickets type 1 is caused by mutations in *CYP27B1* encoding 25(OH)D-1α-hydroxylase [6]. This enzyme converts 25(OH)D into $1,25(OH)_2D$. *Vitamin D receptor (VDR)* is mutated in patients with vitamin D-dependent rickets type 2 [7]. In patients with these vitamin D-dependent rickets, both hypophosphatemia and hypocalcemia are observed. Because hypocalcemia causes secondary hyperparathyroidism resulting in reduced renal tubular phosphate reabsorp-

tion, both impaired intestinal phosphate absorption and increased renal phosphate excretion contribute to the development of hypophosphatemia in these patients. Vitamin D deficiency is diagnosed by low serum 25(OH)D levels. Some patients with vitamin D deficiency also present secondary hyperparathyroidism. Because parathyroid hormone (PTH) stimulates conversion of 25(OH)D into $1,25(OH)_2D$, patients with vitamin D deficiency can present various levels of $1,25(OH)_2D$. Therefore, vitamin D deficiency cannot be diagnosed by $1,25(OH)_2D$ levels.

Gastrectomy or enterectomy can cause osteomalacia [8]. It is possible that patients with osteomalacia after these surgeries are not properly diagnosed and treated. The causes for osteomalacia in these patients may be multifactorial including vitamin D deficiency and impaired mineral absorption. While liver cirrhosis or chronic liver disease was described as one of causes for osteomalacia, osteoporosis seems to be much commoner than osteomalacia in these patients [9].

There are many causes for renal phosphate wasting. About 80-90% phosphate filtered from glomeruli is absorbed in renal proximal tubules. Type 2a and 2c sodium-phosphate cotransporters mediate physiological phosphate reabsorption in proximal tubules [10]. Renal phosphate wasting can be observed in patients with Fanconi syndrome and some form of renal tubular acidosis. In addition, hereditary hypophosphatemic rickets with hypercalciuria is caused by mutations in *SLC34A3* which encodes type 2c sodium-phosphate cotransporter [11, 12]. Dent disease is characterized by low molecular weight proteinuria, hypophosphatemia and nephrolithiasis. This disease is caused by mutations in *CLC5* encoding a chloride channel [13]. In addition to these intrinsic renal tubular defect, excessive actions of FGF23 cause hypophosphatemia with enhanced renal phosphate excretion. FGF23 suppresses renal tubular phosphate reabsorption by reducing the expression of type 2a and 2c sodium-phosphate cotransporters [14]. In addition, FGF23 decreases serum $1,25(OH)_2D$ level by altering the expression levels of vitamin D-metabolizing enzymes [14]. Therefore, FGF23 reduces serum phosphate by suppressing renal phosphate reabsorption and inhibiting intestinal phosphate absorption through lowering $1,25(OH)_2D$.

After the identification of FGF23, it became clear that patients with some kinds of hypophosphatemic rickets/osteomalacia such as X-linked hypophosphatemic rickets (XLH) and tumor-induced osteomalacia (TIO), a rare paraneoplastic syndrome, show high circulatory levels of FGF23 [15, 16]. In contrast, FGF23 levels in patients suffering from other causes for rickets/osteomalacia including vitamin D deficiency were rather low [5]. Together with the biological activities of FGF23 as mentioned above, these results indicated that FGF23 is the humoral factor that causes hypophosphatemia in patients with several diseases including XLH and TIO. Since then, other hypophosphatemic diseases have also been shown to be associated with high FGF23 levels [17] (Table 2). It is believed that FGF23 production in bone is enhanced in most patients with these FGF23-related hypophosphatemic diseases except for TIO. However, the precise mechanisms of overproduction of FGF23 and the actions of gene products responsible for genetic FGF23-related hypophosphatemic diseases are largely unknown.

Table 2 FGF23-related hypophosphatemic rickets/osteomalacia

X-linked dominant hypophosphatemic rickets/osteomalacia (XLH)
Mutations in *PHEX* gene
Autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR)
Mutations in *FGF23* gene
Autosomal recessive hypophosphatemic rickets/osteomalacia 1 (ARHR1)
Mutations in *DMP1* gene
Autosomal recessive hypophosphatemic rickets/osteomalacia 2 (ARHR2)
Mutations in *ENPP1* gene
Hypophosphatemic disease with dental anomalies and ectopic calcification
Mutations in *FAM20C* gene
McCune-Albright syndrome/fibrous dysplasia
Linear sebaceous nevus syndrome
Tumor-induced rickets/osteomalacia
Hypophosphatemic rickets/osteomalacia by saccharated ferric oxide or iron polymaltose…

PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome; DMP1, dentin matrix protein 1; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; FAM20C, family with sequence similarity 20, member C

Diagnostic Criteria (Table 3)

Because the clinical presentations of rickets and osteomalacia are quite different, it is not practical to prepare one kind of diagnostic criteria for both of these diseases. Therefore, we propose the following diagnostic criteria (Table 3). Rickets is basically diagnosed by the presence of rachitic changes of bones such as cupping and fraying of metaphysis, and widening of epiphyseal plate observed by X-ray. In addition, high

alkaline phosphatase is characteristic for diseases with impaired mineralization. We believe that these two laboratory findings are essential to make a diagnosis of rickets or suspect the presence of this disease. In addition, patients with rickets usually show hypophosphatemia or hypocalcemia, and some clinical signs. However, it is possible that patients show no clinical signs especially when the diagnosis of rickets is established in the early phase of the disease. This can happen in family members of affected patients with rickets from genetic causes. Therefore, we propose that patients with all these four findings (a to d in Table 3A) are regarded to have definite rickets, and subjects who lack either hypophosphatemia or hypocalcemia, or clinical signs (c or d) as possible rickets.

In contrast to rickets, there is no single laboratory test which strongly suggests the presence of osteomalacia. However, patients with osteomalacia usually show either hypophosphatemia or hypocalcemia. High alkaline phosphatase is also seen in patients with osteomalacia. While almost all alkaline phosphatase activity in blood derives from bone in children, there is significant contribution from liver and other organs in adults. We believe that either hypophosphatemia or hypocalcemia, and high bone alkaline phosphatase are essential to make a diagnosis of osteomalacia or suspect this disease. In addition, patients with osteomalacia may show some clinical symptoms, low bone mineral density (BMD), multiple uptakes by bone scintigraphy or Looser's zone. However, patients may not show these findings when osteomalacia is suspected early in the course of the disease as in the case of rickets. Therefore, we propose that patients with all these five findings (a to e in Table 3B) are regarded to have definite osteomalacia, and subjects who lack either clinical symptoms, low BMD or abnormal imaging findings (c to e) as possible osteomalacia.

Drugs that inhibit mineralization can induce rickets or osteomalacia without changing serum calcium or phosphate levels. In addition, several diseases need to be discriminated form rickets/osteomalacia (Table 4). Diseases shown in Table 4 can mimic some features of rickets or osteomalacia. On the other hand, most of these diseases cannot be diagnosed as rickets or osteomalacia using the diagnostic criteria shown in Table 3. The exceptions are multiple metastases, primary hyperparathyroidism and renal osteodystrophy. Osteoblastic bone metastases can cause either hypocalcemia or hypophosphatemia, high bone alkaline phosphatase, bone pain and multiple uptakes by bone scintigraphy. This possibility need to be considered before finalizing the diagnosis of osteomalacia. Patients with primary hyperparathyroidism may also show hypophosphatemia, high bone alkaline phosphatase, bone pain, low BMD and multiple uptakes by bone scintigraphy. However, hypercalcemia which is rare in

Table 3 Diagnostic criteria for rickets and osteomalacia

A. Rickets
a) Rachitic changes in X-ray (Cupping and fraying of metaphysis, widening of epiphyseal plate)
b) High alkaline phosphatase
c) Hypophosphatemia or hypocalcemia[1)]
d) Clinical signs
Bone deformities such as genu varum and valgum, abnormal spinal curvature, craniotabes, open fontanels, rachitic rosary, joint swelling
Definite rickets
Patients who have a) to d)
Possible rickets
Patients who show a), b) and one of c) or d)
B. Osteomalacia
a) Hypophosphatemia or hyocalcemia[1)]
b) High bone alkaline phosphatase
c) Clinical symptoms
Muscle weakness or bone pain
d) Low BMD
Less than 80% of YAM
e) Abnormal imaging findings
Multiple uptakes by bone scintigraphy or Looser's zone by X-ray
Definite osteomalacia
Patients who have a) to e)
Possible osteomalacia
Patients who show a), b) and two of c) to e)

[1)] Does not apply to patients with inhibitors of mineralization

Table 4 Diseases that need to be discriminated form rickets/osteomalacia

Low BMD
Osteoporosis, Renal osteodystrophy, Primary hyperparathyroidism
Bone deformity
Skeletal dysplasia
Bone pain
Polymyalgia rheumatica, Ankylosing spondylitis
Muscle weakness
Neuromuscular diseases
Multiple uptakes by bone scintigraphy
Multiple metastases
Rachitic change
Hypophosphatasia
High bone alkaline phosphatase
Primary hyperparathyroidism, Renal osteodystrophy, Multiple metastases

patients with osteomalacia is usually present in patients with primary hyperparathyroidism. Patients with renal osteodystrophy may show hypocalcemia, high bone alkaline phosphatase, bone pain, low BMD and multiple uptakes by bone scintigraphy. On the other hand, hyperphosphatemia rather than hypophosphatemia is usually observed in patients with renal osteodystrophy.

Hypophosphatasia is caused by mutations in *TNALP* which encodes tissue-nonspecific alkaline phosphatase [18]. This disease is also characterized by impaired mineralization because alkaline phosphatase converts pyrophosphate with potent inhibitory effects on mineralization into phosphate. In this sense, hypophosphatasia can be regarded as one cause for rickets. However, patients with hypophosphatasia show low alkaline phosphatase levels in contrast to those with rickets from other causes. Therefore, hypophosphatasia was treated as a disease that should be discriminated from rickets rather than a cause for rickets to avoid confusion in this proposal.

Differential Diagnosis of Causes for Rickets/Osteomalacia (Table 5, Fig. 1)

After establishing the diagnosis of rickets or osteomalacia, it is necessary to find the exact cause for these diseases. Table 5 summarizes typical biochemical changes observed in patients with various causes for rickets and osteomalacia. Vitamin D deficiency is defined by low 25(OH)D levels as mentioned before. Therefore, patients with low 25(OH)D levels are theoretically considered to have vitamin D deficient rickets or osteomalacia. However, vitamin D deficiency or insufficiency is quite common even in general population [19] and it is possible that low 25(OH)D levels can be observed in patients with other causes for rickets or osteomalacia. Actually, investigation of 25(OH)D levels in patients with vitamin D deficient rickets and XLH indicated that there was overlap of serum 25(OH)D levels in these patients. In contrast, FGF23 completely discriminated patients with vitamin D deficient rickets and XLH [20]. From these results, we propose a flowchart for differentiating various causes for rickets and osteomalacia (Fig. 1). In patients with hypophosphatemic rickets/osteomalacia, high FGF23 levels indicate FGF23-related hypophosphatemic diseases (Table 2). Vitamin D deficient rickets/osteomalacia is diagnosed after ruling out FGF23-related hypophosphatemic diseases, phosphate depletion and other causes of renal tubular phosphate wasting. Vitamin D-dependent rickets 1 and 2 can be differentiated by $1,25(OH)_2D$ levels. HHRH is also characterized by high $1,25(OH)_2D$ levels. In normophosphatemic patients, drugs that inhibit mineralization and vitamin D deficiency should be considered. It should be also noted that patients with vitamin D deficiency may not show frank hypophosphatemia or hypocalcemia. It is possible that serum phosphate and calcium remain in the low normal range in these patients.

Discussion

Rickets and osteomalacia are not common diseases. However, quality of life of the affected patients can be severely compromised. For example, some untreated patients with TIO can become completely bedridden because of severe muscle weakness and bone pain. Short stature and bone deformities are big problems for patients with rickets. However, patients with rickets or osteomalacia can be completely cured or at least

Table 5 Typical biochemical findings in patient with various causes of rickets/osteomalacia

	Serum Ca	Serum Pi	TmP/ GFR	BAP	$1,25(OH)_2D$	25(OH)D	FGF23
FGF23-related hypophosphatemic disease	↓→	↓	↓	↑	↓→	→	⇧
Phosphate depletion	→	↓	↑	↑	→↑	→	↓→
Fanconi syndrome	→	↓	↓	↑	↓→	→	↓→
Vitamin D-dependent rickets type 1	↓	↓	↓	↑	↓	→	↓→
Vitamin D-dependent rickets type 2	↓	↓	↓	↑	↑	→	↓→
HHRH	→	↓	↓	↑	↑	→	↓→
Vitamin D deficiency	↓→	↓→	↓→	↑	→↑↓	⇩	↓→
Drugs that inhibit mineralization	→	→	→	↑	→	→	→

⇩⇧: Specific laboratory tests for each category.

Pi, phosphate; TmP/GFR, tubular maximum transport of phosphate per glomerular filtration rate; BAP, bone alkaline phosphatase; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; HHRH, hereditary hypophosphatemic rickets with hypercalciuria

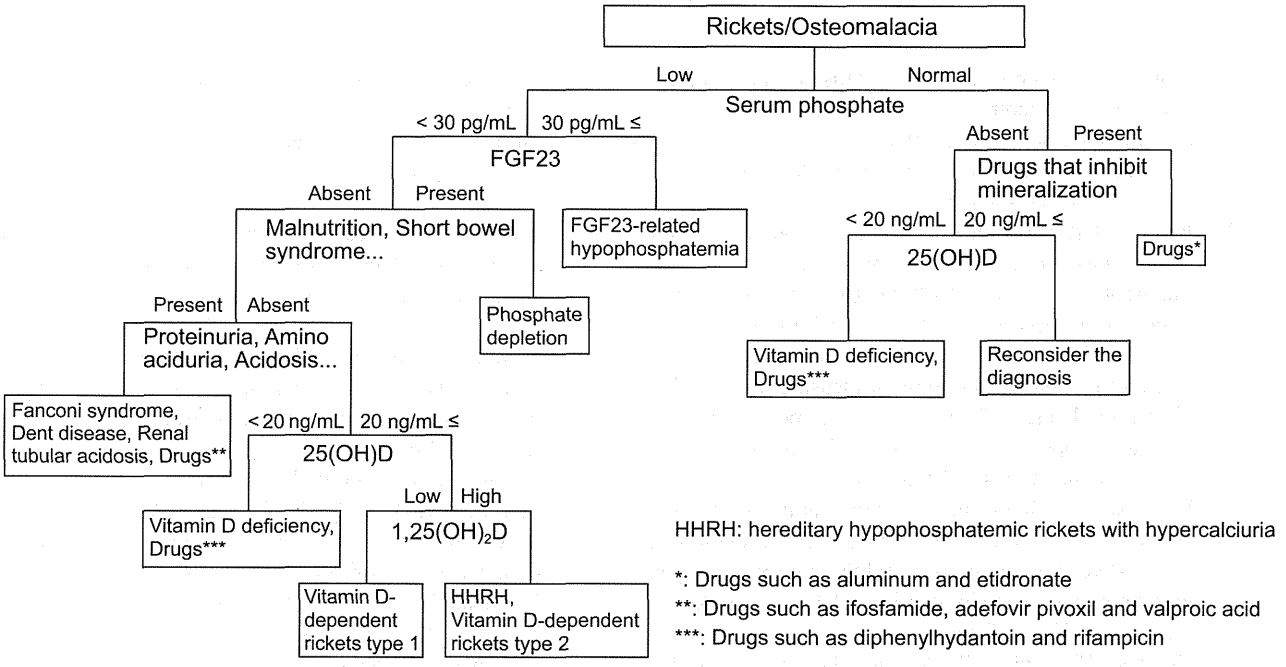

Fig. 1 A flowchart for the differential diagnosis of causes for rickets/osteomalacia
The causes for rickets and osteomalacia can be identified by several clinical findings and laboratory tests.

respond to treatment when properly diagnosed and treated according to the specific causes. Therefore, we here listed causes for rickets and osteomalacia, and proposed the diagnostic criteria and a flowchart for the differential diagnosis of various causes for these diseases.

There are several limitations in the proposed diagnostic criteria. These criteria are not created by retrospective review of clinical presentations of a large number of patients, but proposed by several researchers and clinicians based on their experiences. Therefore, the validity of these criteria and the flowchart needs to be examined in the future studies. However, without any diagnostic criteria, it would be difficult for general medical professionals to correctly diagnose not-so-common illnesses. We hope that this proposal will become a momentum for propagation of proper knowledge about rickets and osteomalacia, and for accumulation of more clinical data for future revision of the criteria. In addition, there was a discussion about hypophosphatasia among us. This disease can be considered to be one cause of rickets. However, if hypophosphatasia is included in the causes for rickets, high alkaline phosphatase cannot be used as one of criteria for the diagnosis of rickets, and the flowchart for the differential diagnosis of various causes needs to be more complex. Because hypophosphatasia is rarer than other causes for rickets such as vitamin D deficiency and XLH, and the easily usable diagnostic criteria for rickets and osteomalacia were planned, hypophosphatasia was not included as a cause for rickets in this proposal. Finally, measurements of FGF23 and 25(OH)D are not covered by medical insurance in Japan now and are not routine laboratory tests. In contrast, these measurements are available in several commercial and research laboratories. We hope that this proposal will contribute to some extent to the future coverage of these measurements by medical insurance in Japan.

In summary, we have created a diagnostic criteria and a flowchart for the differential diagnosis of various causes for rickets/osteomalacia. We hope that these criteria and flowchart are clinically useful for the proper diagnosis and management of patients with these diseases.

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Disclosure

The authors declare no conflicts of interest.

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ORIGINAL

Nationwide survey of fibroblast growth factor 23 (FGF23)-related hypophosphatemic diseases in Japan: prevalence, biochemical data and treatment

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Abstract. A nationwide epidemiologic survey of fibroblast growth factor 23 (FGF23)-related hypophosphatemic diseases was conducted in 2010 to clarify the prevalence and the clinical presentations of the disorders. A questionnaire inquiring the experience of patients with these diseases was sent to randomly selected hospitals throughout Japan. The estimated annual incidence of the diseases was 117 cases (95% CI 75 - 160), 55 males (95% CI 30 - 81) and 62 females (95% CI 40 - 84). Tumor-induced osteomalacia (TIO) and X-linked hypophosphatemic rickets (XLH) were the most prevalent causes of acquired and genetic FGF23-related hypophosphatemic diseases, respectively. The estimated incidence of XLH was about 1 in 20,000. We have also collected clinical data of the patients by a secondary survey. These patients showed FGF23 levels of above 30 pg/mL by intact assay in the presence of hypophosphatemia. While complete resection of responsible tumors improved biochemical abnormalities in patients with TIO, treatment with phosphate and/or active vitamin D_3 did not normalize serum phosphate and tubular maximum transport of phosphate in patients with XLH. Our results suggest that there is no racial difference in the incidence of XLH. While FGF23 measurement is useful for the diagnosis of FGF23-related hypophosphatemic diseases, the better management is necessary especially for patients with genetic hypophosphatemic rickets caused by excessive actions of FGF23.

Key words: Hypophosphatemia, Rickets, Osteomalacia, FGF23

FIBROBLAST GROWTH FACTOR 23 (FGF23) is a hormone produced by bone and reduces serum phosphate by inhibiting proximal tubular phosphate reabsorption and intestinal phosphate absorption through decreasing serum 1,25-dihydroxyvitamin D [1]. It has been shown that excessive actions of FGF23 cause several kinds of FGF23-related hypophosphatemic diseases such as X-linked hypophosphatemic rickets (XLH), autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets (ARHR) 1 and 2, tumor-induced osteomalacia (TIO), hypophosphatemic disease associated with McCune-Albright syndrome/fibrous dysplasia and hypophosphatemia caused by intravenous administration of saccharated ferric oxide [2]. However, there have been no epidemiological studies concerning these FGF23-related hypophosphatemic diseases. In addition, the clinical presentations of and the treatment methods for these diseases are not sufficiently

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Table 1 Diseases caused by excess actions of FGF23

Diseases	Responsible gene
X-linked hypophosphatemic rickets	*PHEX*
Autosomal dominant hypophosphatemic rickets	*FGF23*
Autosomal recessive hypophosphatemic rickets 1, 2	*DMP1, ENPP1*
Hypophosphatemic disease associated with McCune-Albright syndrome/fibrous dysplasia	*GNAS*
Tumor-induced rickets/osteomalacia	
Hypophosphatemic disease caused by intravenous administration of saccharated ferric oxide	
Linear nevus sebaceous syndrome	

PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome; FGF23, fibroblast growth factor 23; DMP1, dentine matrix protein 1; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; GNAS, guanine nucleotide binding protein alpha-stimulating

described mainly because these are rare diseases. In contrast, these epidemiological and clinical data are essential for establishing correct diagnostic criteria and appropriate treatment. Therefore, The Hormone Receptor Abnormality Research Committee and The Epidemiological Study Group of Specified Rare and Intractable Diseases supported by Ministry of Health, Labour and Welfare of Japan conducted a nationwide survey of FGF23-related hypophosphatemic diseases in Japan in 2010.

Patients and Methods

This study was approved by the ethics committee of Tokushima University. The primary and the secondary surveys were conducted. The purpose of the primary survey was to estimate the number of patients with hypophosphatemic diseases caused by excess actions of FGF23. In addition, the secondary survey was done to clarify the clinical features and course of these patients. A nationwide primary mail survey was conducted in 2010. Patients who visited hospitals because of suspected FGF23-related hypophosphatemic diseases in 2009 were the targets of the primary survey. According to the Nationwide Epidemiologic Survey Manual issued by The Epidemiological Study Group of Specified Rare and Intractable Diseases, we selected three medical departments of internal medicine (including endocrinology), orthopedics and pediatrics as candidates for the survey. Study hospitals were selected randomly from the list of all hospitals in Japan. The selection rate was decided according to the stratification classified by the number of beds in the hospitals; the more beds a hospital has, the higher the probability to be selected. The selection rate was 100% for hospitals with more than or equal to 500 beds and medical university hospitals, whereas only 5% of hospitals with less than 100 beds were selected at random. After the selection of the study hospitals, we sent questionnaires to the three departments of the selected hospitals with a list of diseases known to be caused by excess actions of FGF23 at that time (Table 1). The questionnaires asked the number of new patients with these FGF23-related hypophosphatemic diseases in 2009 and the total number of patients between 2005 and 2009. When some department with the hypophosphatemic diseases responded, the second mail survey questionnaire asking detailed clinical features including the diagnosis, biochemical data and treatment for each patient was sent to that department. All blood and urine sample were taken at fasting. We also offered to measure FGF23 in hypophosphatemic patients if FGF23 levels were unknown to confirm the diagnosis of FGF23-related hypophosphatemic diseases.

Considering the selection rate and the response rate to the survey, we estimated the total numbers of patients with FGF23-related hypophosphatemic diseases as follows.

The formula for the estimation of the patient number in each stratum is;
The estimated number of patients
= reported number of patients / (selection rate x response rate)
And the numbers of patients for each stratum were summed up. Ninety-five percent confidence intervals were calculated with an assumption of multinomial hypergeometric distribution [3].

Results

From 14,100 departments of internal medicine, orthopedics and pediatrics all over Japan, 2895 (20.5%) study departments were selected at random for the primary survey. Replies were obtained from 1149

Table 2 Results of the secondary survey of FGF23- related hypophosphatemic diseases in Japan

Diagnosis	Number		Ages at diagnosis
TIO	male	19	27 - 64
	female	16	16 - 79
	total	35	
Genetic hypophosphatemic diseases	male	15	0 - 7
(XLH, ADHR , ARHR1, 2)	female	26	0 - 7
	total	41	
Saccharated ferric oxide induced	male	3	58 - 87
	female	3	43 - 54
	total	6	
Linear nevus sebaceous syndrome	male	0	
	female	2	1 - 5
	total	2	
	total	84	

TIO, tumor-induced osteomalacia; XLH, X-linked hypophosphatemic rickets; ARHR, autosomal recessive hypophosphatemic rickets, ADHR, autosomal dominant hypophosphatemic rickets

Table 3 Results of biochemical parameters in the secondary survey

Diagnosis	Serum P (mg/dL)	TmP/GFR (mg/dL)	FGF23 (range; pg/mL)
TIO	1.74 ± 0.35	1.31 ± 0.37	1304.1 ± 3660.3 (44.0 – 18286.4)
Genetic hypophosphatemic diseases	2.47 ± 0.58	2.17 ± 0.71	325.2 ± 1086.3 (40.3 – 4540.0)
Saccharated ferric oxide	1.12 ± 0.18	0.82 ± 0.68	277.5 ± 242.7 (30.2 – 654.0)
Linear nevus sebaceous syndrome	2.03 ± 0.31	1.82 ± 0.48	92.6

TmP/GFR, tubular maximum transport of phosphate per glomerular filtration rate; FGF23, fibroblast growth factor 23; TIO, tumor-induced osteomalacia

(39.7%) departments and 95 departments reported the presence of patients with FGF23-reltaed hypophosphatemic diseases. Total number of the patients was 311 per 5 years and the number of new patients in 2009 was 63. The annual incidence of FGF23-related hypophosphatemic diseases in Japan was estimated to be 55 in males (95% confidence interval 30 – 81) and 62 in females (40 – 84) resulting in 117 (75 – 160) by summing up the estimated number of patients in each stratum according to the hospital size.

On the second survey, 36 departments (37.9%) replied and clinical data of 84 patients were collected. The demographic information of these patients is shown in Table 2. There were 35 patients with TIO and the diagnosis was confirmed by resection of the responsible tumors in all of these patients. The numbers of male and female patients with TIO were almost the same. The ages at the time of diagnosis were quite variable. There were 41 patients with genetic hypophosphatemic rickets, 36 patients with XLH, 3 patients with ARHR1, 1 patient each with ARHR2 and ADHR. The number of female patients was larger than that of male patients. All the patients with genetic hypophosphatemic rickets were diagnosed at seven years old or younger. Of these, the diagnosis was confirmed by genetic analysis in 15 patients. In addition, there were 6 hypophosphatemic patients by intravenous administration of saccharated ferric oxide. Serum phosphate increased in all of these patients after the cessation of the causative drug. There were 2 hypophosphatemic female patients caused by linear nevus sebaceous syndrome.

Table 3 shows baseline biochemical data by the secondary survey. The mean phosphate level and standard deviation in patients with TIO was 1.74 ± 0.35 mg/dL, and TmP/GFR (tubular maximum transport of phosphate per glomerular filtration rate) was 1.31 ± 0.37 mg/dL. Serum phosphate in patients with genetic hypophosphatemic diseases was 2.47 ± 0.58 mg/dL and at the lower limit of the reference range for adults. However, this value was considered to be low because more than half of the patients in this group were children. Serum phosphate and TmP/GFR were 1.12 ± 0.18 and 0.82 ± 0.68 mg/dL, respectively, in hypophosphatemic patients caused by saccharated ferric oxide. FGF23 concentrations measured by full-length assay were above 30 pg/mL in all the patients [4]. However, FGF23 levels were quite variable as shown in Table 3. At baseline, no patients had renal disorder or elevation

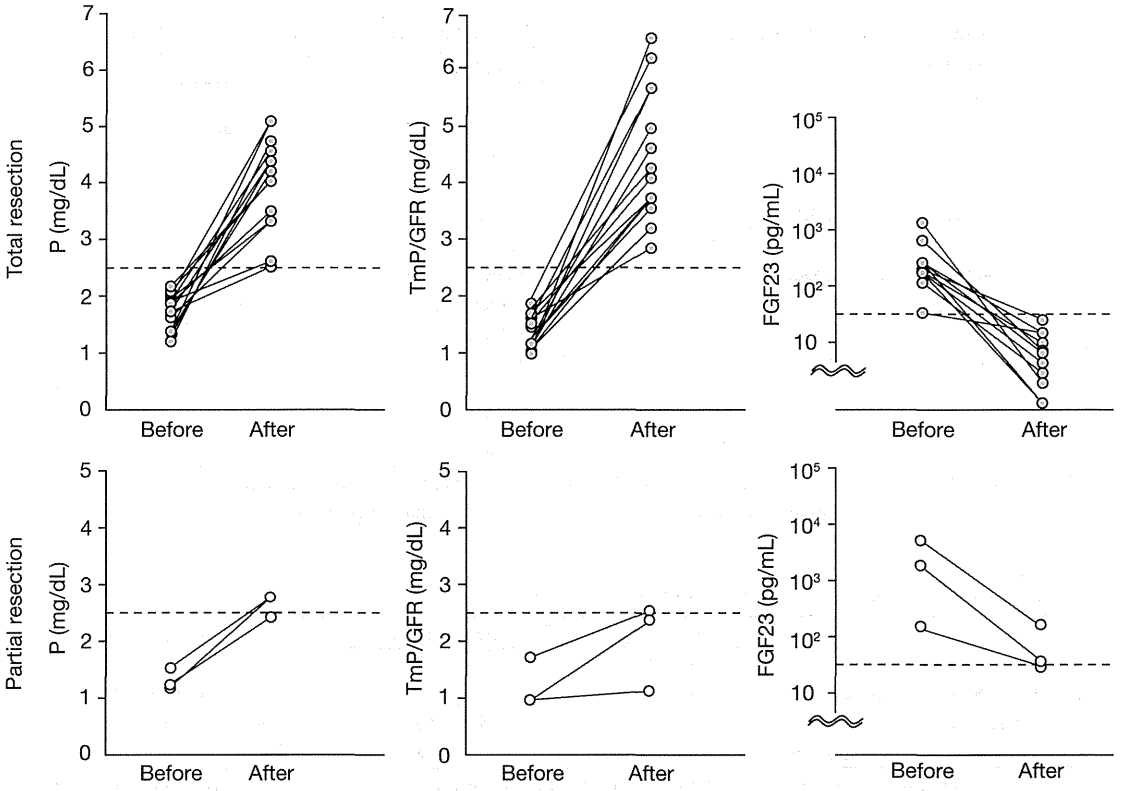

Fig. 1 Serum phosphate, TmP/GFR and FGF23 levels before and after resection of responsible tumors in patients with tumor-induced osteomalacia. The closed circles indicate patients cured by the surgery with complete resection of responsible tumors (14 patients) and the open ones show patients whose responsible tumors could not be completely resected (3 patients). The broken lines indicate the lower limit of the reference range of serum phosphate and TmP/GFR, and the upper limit of the reference range of FGF23. TmP/GFR: tubular maximum transport of phosphate per glomerular filtration rate.

of serum creatinine.

We also obtained the biochemical data of 17 patients with TIO both before and after the resection of responsible tumors. Of these, the responsible tumors were completely removed in 14 patients and the results were shown in the upper panel of Fig. 1. Serum phosphate, TmP/GFR and FGF23 normalized in all of the 14 patients. On the other hand, TmP/GFR did not normalize and FGF23 remained high or in the high normal range in 3 patients whose tumors could not be completely removed.

We collected biochemical data of 7 children and 4 infants with XLH both before and after the initiation of treatment with phosphate and/or active vitamin D_3. As shown in Fig. 2, serum phosphate and TmP/GFR did not normalize despite the initiation of the treatment with oral phosphate and/or active vitamin D_3. We also obtained the biochemical data of 7 adults, 4 children and 1 infant who already had been treated with active vitamin D_3 and/or phosphate at baseline. Their treatment period was at least 2 years and the serum phosphate levels were under the lower limit of the reference range at baseline while on treatment.

Finally, we assessed the clinical course of hypophosphatemic disease caused by intravenous administration of saccharated ferric oxide. As shown in Fig. 3, the mean serum phosphate and TmP/GFR during the administration of saccharated ferric oxide were quite low. However, after the cessation of the responsible drug, serum phosphate and TmP/GFR increased to the reference range and FGF23 decreased.

Discussion

The results of this nationwide survey indicate that estimated annual incidence was about 100 patients with FGF23-related hypophosphatemic diseases in Japan. Of these, TIO and XLH were the most prevalent causes of acquired and genetic FGF23-related hypophosphatemic diseases, respectively. The num-

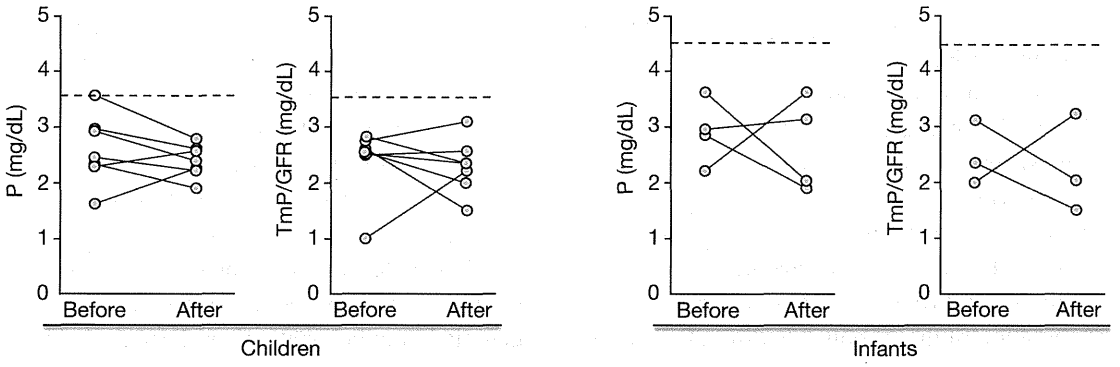

Fig. 2 Serum phosphate and TmP/GFR in patients with XLH before and after the treatment with phosphate and/or active vitamin D_3. The broken lines indicate the lower limit of the reference range of serum phosphate and TmP/GFR. TmP/GFR: tubular maximum transport of phosphate per glomerular filtration rate.

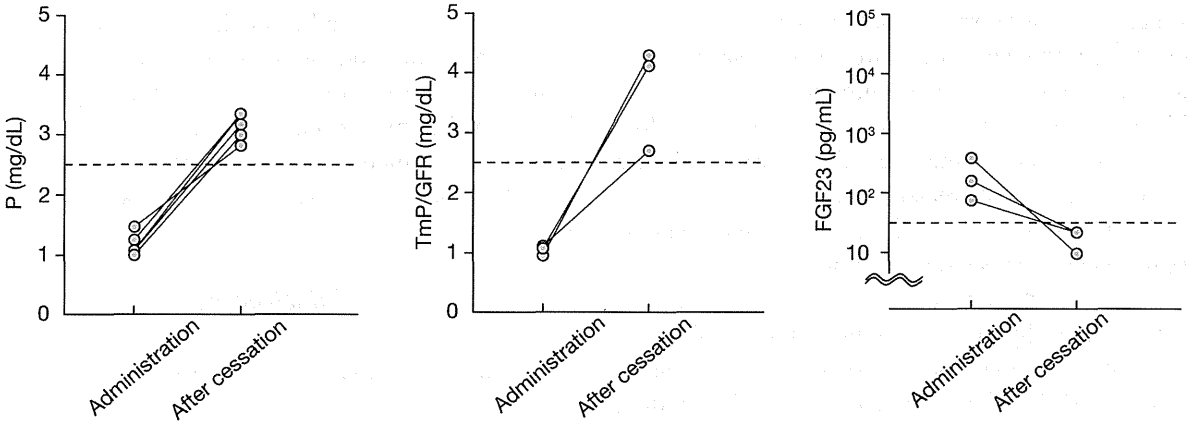

Fig. 3 Serum phosphate, TmP/GFR and FGF23 levels during administration and after cessation of saccharated ferric oxide in hypophosphatemic patients. The broken lines indicate the lower limit of the reference range of serum phosphate and TmP/GFR, and the upper limit of the reference range of FGF23. TmP/GFR: tubular maximum transport of phosphate per glomerular filtration rate.

bers of patients with TIO and XLH were similar indicating that there are about 50 new patients with XLH in Japan. Considering that the number of live births is almost 1,000,000 per year in Japan, the incidence of XLH is estimated to be about 1 in 20,000. This number is exactly the same as that reported in North America suggesting that these is no big racial difference in the incidence of XLH [5].

Previously, we have proposed that FGF23 levels above 30 pg/mL by intact FGF23 assay in the presence of hypophosphatemia indicate the diseases caused by excessive actions of FGF23 such as TIO and XLH [6]. In this secondary survey, all the patients showed serum FGF23 above 30 pg/mL while serum phosphate was low or low normal range. Therefore, the validity of the previous proposal was confirmed by this survey.

TIO occurs mainly in adults with a mean age of 45 ± 16 years [7] and men and women appear to be equally affected [8]. Our data indicate almost the same epidemiological results. In the present study, all TIO patients with successful resection of responsible tumors showed improvement in serum phosphate, TmP/GFR and FGF23. However, serum phosphate and/or TmP/GFR did not normalize in patients with remaining tumors. These results indicate that complete resection of responsible tumors is necessary to cure patients with TIO.

We obtained biochemical parameters both before and after the treatment from 11 patients with XLH and also corrected serum phosphate data from 12 XLH patients who already had been treated with active vitamin D_3 and/or phospate. These data indicate that the treatment with oral phosphorus and/or active vitamin D_3 did not normalize serum phosphate and TmP/GFR as these drugs do not enhance proximal tubular phosphate reabsorption. Therefore, novel therapeutic approach to inhibit FGF23 actions is necessary for

patients with FGF23-related hypophosphatemic diseases including XLH. A clinical trial using anti-FGF23 antibody has already demonstrated that the inhibition of FGF23 activity results in increased serum phosphate and enhanced renal tubular phosphate reabsorption in adult patients with XLH [9].

Serum phosphate was quite low and around 1 mg/dL in patients treated with saccharated ferric oxide. However, high FGF23 and hypophosphatemia improved after the cessation of the responsible drug. Saccharated ferric oxide is widely used for iron deficiency anemia in Japan and there are several reports of hypophosphatemic osteomalacia by this drug [10]. It is likely that long-lasting hypophosphatemia causes symptomatic osteomalacia. Therefore, it would be prudent to monitor serum phosphate in patients treated with saccharated ferric oxide even if they are asymptomatic.

The major drawback of the study was the low response rate even in the secondary survey. There seem to be a couple of reasons for the low reply rate. First, the importance of FGF23 in the development of hypophosphatemic diseases may not have yet been well recognized. In addition, while we offered to measure FGF23 levels in hypophosphatemic patients if necessary, it is possible that doctors could not easily evaluate serum FGF23 in clinical practice. Notwithstanding this limitation, the survey produced the same estimated incidence of XLH as the previous report [5].

In conclusion, this is the first epidemiological survey concerning FGF23-related hypophosphatemic diseases. Our results suggest that the incidence of XLH is similar in Japan to that in North America. While FGF23 measurement is useful for the diagnosis of FGF23-related hypophosphatemic diseases, the better management is necessary especially for patients with genetic hypophosphatemic rickets caused by excessive actions of FGF23.

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Disclosure

The authors declare no conflicts of interest.

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CASE REPORT

Vitamin D-mediated hypercalcemia in multicentric Castleman's disease

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Abstract Multicentric Castleman's disease (MCD) is a rare lymphoproliferative disorder, which represents various symptoms caused by the hyperproduction of interleukin-6 (IL-6). However, case studies of MCD accompanied by hypercalcemia have rarely been reported thus far. A 78-year-old male had generalized fatigue, and his laboratory data revealed elevated serum calcium (Ca) and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] levels (11.5 mg/dl and 80 pg/ml, respectively), while the serum intact parathyroid hormone level was low (4 pg/ml). Computed tomography showed multicentric lymphadenopathy. The serum IL-6 level was elevated (20.7 pg/ml), and pathological examination of a supraclavicular lymph node specimen led us to diagnose MCD. Moreover, immunostaining analysis showed that vitamin D-activating enzyme 25-hydroxyvitamin D 1-alpha-hydroxylase was expressed in lymph node macrophages. Prednisolone treatment improved the hypercalcemia and decreased the levels of 1,25$(OH)_2$D and IL-6. We first reported a case of vitamin D-mediated hypercalcemia in MCD.

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Keywords Hypercalcemia · Castleman disease · Vitamin D · 25-Hydroxyvitamin D 1-alpha-hydroxylase · Prednisolone

Introduction

Multicentric Castleman's disease (MCD) is known to be an uncommon lymphoproliferative disorder accompanied by generalized peripheral and visceral lymphadenopathy as well as multiorgan involvement [1, 2]. Various symptoms, such as fever, fatigue and generalized lymphadenopathy, are caused by the hyperproduction of interleukin-6 (IL-6) in MCD [3]. However, the accurate incidence as well as detailed pathophysiologic condition of MCD remains unclear. Although a few cases of MCD accompanied by hypercalcemia have been reported, the underlying mechanism is still unknown. We herein report a case of hypercalcemia in a patient with MCD showing the expression of 25-hydroxyvitamin D 1-alpha-hydroxylase in lymphadenopathy.

Case report

A 78-year-old Japanese male had taken 2 mg/day prednisolone because of autoimmune hemolytic anemia (AIHA) and idiopathic thrombocytopenic purpura (ITP) for 2 years. Because hypercalcemia (serum calcium 14.8 mg/dl) and renal failure were found, he received treatments with elcatonin and bisphosphonate 2 month ago. Then, he was referred to our department for detailed examination and treatment.

The patient had symptoms and abnormal physical findings, such as generalized fatigue, loss of appetite and

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body weight, and enlargement of superficial lymph nodes. However, he did not have any bone symptoms or abnormal imaging findings on the bone X-ray test or bone scintigraphy. Laboratory data on admission (Table 1) showed that the serum calcium level corrected with albumin was elevated (11.5 mg/dl), the phosphorus level was within the normal range (2.9 mg/dl), and renal failure was observed (serum creatinine 2.35 mg/dl and estimated glomerular filtration rate 20.0 ml/min/BSA), while the intact parathyroid hormone (int-PTH) level was decreased (4 pg/ml, normal range 10–65 pg/ml). The PTH-related peptide (PTHrP) value was not elevated (<1.1 pmol/l). The serum level of $1,25(OH)_2D$ was elevated to 80 pg/ml (normal range 20–60 pg/ml). Bone turnover markers such as bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase-5b were decreased [7.0 μg/l (normal range 13.0–33.9 μg/l) and 157 mU/dl (normal range 170–590 mU/dl), respectively].

Table 1 Laboratory data (serum levels of indices related to calcium metabolism)

Parameter	Value	Normal range
Corrected Ca (mg/dl)	11.5	8.6–10.3
P (mg/dl)	2.9	2.2–4.6
Intact PTH (pg/ml)	4	10–65
Whole PTH (pg/ml)	7.7	9.0–39.0
PTHrP (pmol/l)	<1.1	<1.1
BAP (U/l)	7.0	13.0–33.9
Osteocalcin (ng/ml)	1.9	2.5–13.0
TRACP-5b (mU/dl)	157	170–590
$1,25(OH)_2D$ (pg/ml)	80.0	20.0–60.0
25(OH)D (ng/ml)	23.3	9.0–33.9
Urine Ca (mg/day)	1150	<200

PTH parathyroid hormone, *PTHrP* parathyroid hormone-related peptide, *BAP* bone alkaline phosphatase, *TRACP-5b* tartrate-resistant acid phosphatase 5b, *$1,25(OH)_2D$* 1,25-dihydroxyvitamin D, *25(OH)D* 25-hydroxyvitamin D

The serum IL-6 level was elevated (20.73 pg/ml, normal range <4.0 pg/ml). Computed tomography scans showed that the left supraclavicular, periaortic and bilateral inguinal lymph nodes were enlarged. An examination of a left supraclavicular lymph node specimen showed that there were shrunken germinal centers with concentric expansion of the mantle zones with eosinophils and hyalinization around the vessels, leading to the histologic diagnosis of Castleman's disease. The angiotensin-converting enzyme level was assessed to evaluate sarcoidosis; the test result was within the reference range. Histoplasmosis titers were negative, and the QuantiFERON tuberculin test was negative for *Mycobacterium tuberculosis*. Human immunodeficiency virus screening was also negative. The serum IgG4 level was normal (6 mg/ml, normal range 4–108 mg/ml). These results excluded other granulomatous and infectious diseases. Moreover, an immunostaining analysis showed that the vitamin D-activating enzyme 25-hydroxyvitamin D 1-alpha-hydroxylase was expressed in CD68-positive macrophages of the lymph node specimen (Fig. 1). Based on the diagnosis of MCD, treatment with prednisolone (40 mg/day) was performed. As shown in Fig. 2, prednisolone treatment markedly improved the hypercalcemia and high levels of $1,25(OH)_2D$ and IL-6 as well as the patient's symptoms such as generalized fatigue and lymphadenopathy.

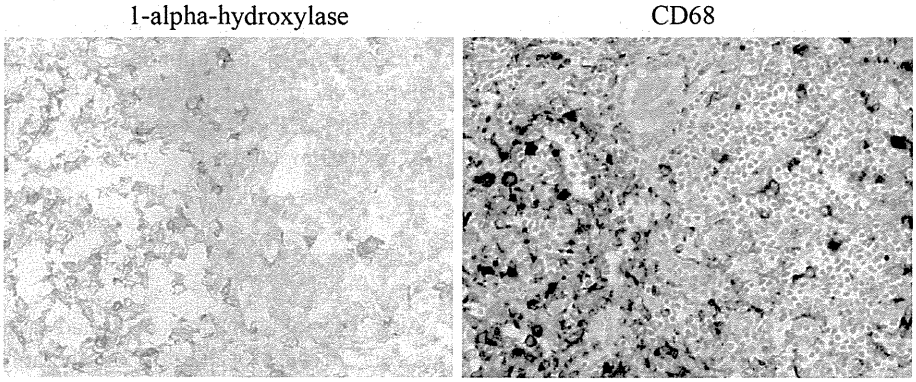

Fig. 1 Immunohistochemical analysis of 25-hydroxyvitamin D 1-alpha-hydroxylase and CD68-positive macrophages in a supraclavicular lymph node. A 4-μm-thick frozen section cut from the surgically excised lymph node was fixed with a 3.7 % formaldehyde-phosphate-buffered saline (PBS) solution for 5 min and successively treated with a mouse monoclonal anti-CYP27B1 antibody (H-9, Santa Cruz Biotechnology Inc.) at 1:100 dilution, followed by visualization with a streptavidin-conjugated mouse IgG staining system. As a negative control, we confirmed no positive staining in the lymph nodes dissected from other patients who underwent cancer surgeries with normal serum calcium levels (data not shown). Normal kidney tissue was used as a positive control. *Left panel* Staining for 25-hydroxyvitamin D 1-alpha-hydroxylase (*brown*) (×200). *Right panel* Staining for CD68 (brown) (×200). Positive staining is shown in *brown color*