

Phosphorus Metabolism

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Abstract

Phosphorus plays a pivotal role in various biological processes. Therefore, a deeper understanding of Phosphorus Homeostasis is essential for management and treatment of conditions causing an imbalance in phosphate metabolism. The widely understood parathyroid hormone (PTH) and vitamin D axis that governs this phosphate homeostasis has been critiqued for its inability to explain a few rare genetic and acquired conditions associated with phosphate imbalance. Such conditions are characterized by normal PTH and activated vitamin D hormone. For example, Tumor Induced osteomalacia, Autosomal Dominant hypophosphatemic rickets, and X-linked hypophosphatemic rickets.

Recent studies of such conditions have led to the discovery of additional factors that play an important role in phosphorus homeostasis. These phosphaturic factors, called "Phosphatonins" include Fibroblast Growth Factor 23 (FGF-23), Fibroblast Growth Factor 7 (FGF7), Frizzled related protein 4 (FRP4), and matrix extracellular phosphoglycoprotein (MEPE). Out of these phosphatonins, FGF-23 has been extensively studied. This article aims to summarize the importance of phosphatonins in hypo- and hyperphosphatemic conditions along with the physiological and clinical importance of such factors. Furthermore, we tried to summarize current knowledge regarding diagnosis and management of such conditions.

Introduction

Phosphorus is the second most abundant essential mineral in the human body after calcium. It not only plays a role in numerous biologic processes, including energy metabolism and bone mineralization, but also provides the structural framework for DNA and RNA. It is synthesized through various biochemical pathways such as glycolysis and beta oxidation. As a part of signal transduction, phosphate is used in cyclic AMP and products of deoxyribonucleoside diphosphates like dADP, dCDP, dGDP, and dUDP [1].

Approximately 80% to 90% of phosphorus is present in the bones and teeth in the form of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) [2]. The remainder is present in extracellular fluid (ECF), soft tissues and erythrocytes. Serum and plasma contain only a small fraction of total body phosphorus in the form of inorganic phosphate, lipid phosphorus, and phosphoric ester phosphorus. Thus, changes in serum phosphate levels do not necessarily reflect the body's total store of phosphorus.

Phosphate Homeostasis

A normal diet provides approximately 20 mg/kg/day of phosphorus of which 16 mg/kg/day is absorbed in the small intestine (predominantly in the jejunum) by both para-cellular and intra-cellular processes. The intra-cellular process is mediated via Sodium-Phosphate co-transport present on villi of small intestine. The para-cellular pathway is a concentration gradient-dependent, passive transport system. Increase in dietary phosphorus leads to an increase in phosphate absorption with little evidence of an upper limit or saturation of absorption process [2]. Three mg/kg/day of phosphorus is secreted into the intestine via pancreatic and intestinal secretions, giving a net phosphorus absorption of around 13 mg/kg/day. Once absorbed, phosphate/phosphorus enters the ECF and circulation where it is taken up by bones, teeth, and other soft tissues via the action of sodium phosphate co-transporters [1]. Three sodium-phosphate co-transporters have been described: NaPi-I, NaPi-II (a, b, and c) and NaPi-III. NaPi-IIb is localized in the small intestine whereas NaPi-IIa and NaPi-IIc are found in the kidneys, with the former responsible for at least 85% of renal phosphate absorption through intracellular

processes (Tables 1 and 2). The rate of reabsorption and mineralization is important in determining the serum phosphorus concentration. Approximately 3 mg/kg/day of phosphorus is exchanged between mineralized bones and the ECF.

Phosphate is filtered freely across the glomerulus-about 13 mg/kg/day in a normal adult-and 60% to 70% of filtered phosphate is reabsorbed in the proximal tubule (with 10% to 15% reabsorbed in the distal tubule). This transport is mediated via a sodium-gradient dependent process and sodium-phosphate co-transporters (type I and type IIa/IIc) on the luminal brush border membrane of the proximal tubules [3]. Seven mg/kg/day of phosphorus is excreted through the feces. Alterations in the expression of co-transporters on the luminal brush border membrane and microvilli of the small intestine determine the rate of phosphate reabsorption.

Parathyroid Hormone (PTH) and a diet high in phosphate result in the endocytosis of these transporters, thus leading to decreased absorption and phosphaturia. PTH binds to specific receptors in the basolateral membrane resulting in the activation of a protein kinase a pathway that leads to phosphorylation of the sodium hydrogen exchange regulatory factor I (NHERF-1), which plays a role in transcriptional regulation of NaPi-IIa. The dissociation of NHERF-1/NaPi-IIa results in endocytosis of NaPi-IIa and decreased reabsorption of phosphate. On the other hand, PTH deficiency and a diet low in phosphate lead to the insertion of transporters in the membrane,

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Received April 12, 2012; Accepted July 20, 2012; Published July 25, 2012

Citation: Raina R, Garg G, Sethi SK, Schreiber MJ, Simon JF, et al. (2012) Phosphorus Metabolism. J Nephrol Therapeutic S3:008. doi:10.4172/2161-0959.S3-008

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resulting in increased phosphate reabsorption [3,4]. Primary or secondary hyperparathyroidism can lead to hypophosphatemia. Vitamin D deficiency (which causes secondary hyperparathyroidism) can also cause decreased gastrointestinal absorption.

This PTH-Vitamin D axis has been used to explain the renal regulation of phosphate, but it fails to explain the mechanism behind isolated renal phosphate wasting syndrome as seen in [5] tumor-induced osteomalacia [6], autosomal recessive hypophosphatemic rickets [2], autosomal dominant hypophosphatemic rickets [2], and x-linked hypophosphatemia [7] disorders characterized by normal PTH levels, the absence of hypocalcemia, and low or normal calcitriol levels. Thus, these disorders do not increase calcitriol levels to cause hypophosphatemia.

Recent studies in phosphorus homeostasis have led to the discovery of other factors, distinct from PTH, that regulate phosphate excretion and reabsorption. Dietary Pi intake [8], dopamine, adrenergic activity and blood pH changes are known to influence plasma Pi concentrations [2]. This finding suggests that there is a unique intestinal factor that is released in response to the luminal phosphate concentration in the intestine, one that mediates the increased fractional excretion of phosphate during short-term increases in dietary phosphate consumption [9].

Phosphatonins

Kumar et al. [10] described a circulating factor that would explain the presence of phosphaturia and altered vitamin D regulation in patients with tumor-induced osteomalacia. Because tumor removal normalizes phosphate metabolism, an unidentified humoral phosphaturic factor had to be responsible for this syndrome. The author called this factor a "phosphatonin". This circulating factor promotes phosphate excretion by decreasing expression of the sodium phosphate co-transporters in the brush border of the renal proximal tubule. Shimada et al. [11] in 2001 officially identified this factor as fibroblast growth factor (FGF-23). Conversely, concentrations of FGF-23 are reduced in patients with tumoral calcinosis, a disorder characterized by hyperphosphatemia, reduced fractional excretion of phosphate and deposits of calcium phosphate in soft tissues [12,13].

Similarly, in Hyp mice, there is a circulating humoral factor, FGF-23, capable of producing hypophosphatemia, which remains uncorrected even in the presence of a healthy transplanted kidney. The fractional excretion of phosphate, in these mice, is not affected by changes in dietary phosphate, PTH, or 1,25 Dihydroxycalciferol [14]. Even in the presence of a very high dose of 1,25 Dihydroxycalciferol (five times that of normal), the fractional excretion remained the same, despite an increase in serum calcium and phosphate levels [11].

In fact, studies have revealed that there are numerous circulating humoral phosphaturic factors besides FGF-23. These include Fibroblast Growth Factor 7 (FGF7), Frizzled related protein 4 (FRP4), and matrix extracellular phosphoglycoprotein (MEPE) [9]. FGF23, however, is the most extensively studied phosphatonin.

FGF23

FGF 23 is a 251 amino acid, 32 kilo-dalton protein that is expressed and secreted predominantly by osteoblasts in bone. It is also expressed in the thymus, lymph nodes, and endothelial cells surrounding venous sinusoids in the bone marrow [15]. The FGF23 gene is located on chromosome 12p13 and has amino acid homology with FGF19 and FGF21. While FGF23 can activate FGF receptors in the kidneys [16],

hypophosphatemia does not result from this activation alone. A trans-membrane protein named "Klotho" shares a common signal transduction pathway with FGF23 and it is believed that tissue *Klotho-FGF23* coexpression is required for signaling activation [17]. It has been shown that Klotho-deficient mice not only develop hyperphosphatemia, hypercalcemia, elevated calcitriol levels, and vascular calcification but they also age prematurely. The protein was named Klotho after one of the three Moirae (Fates) in Greek mythology who controlled the thread of life. In addition to phosphaturia, FGF23 can suppress alpha hydroxylase activity with resulting low calcitriol levels, thus causing negative feedback.

Tissue Targets of FGF23

Co-expression of FGF23 and Klotho is needed for FGF23 activation. Klotho is expressed in several tissues, including the kidney, reproductive tract and brain [17]. The kidney is the principal target, and the principal action of Klotho-FGF23 occurs in the proximal tubule, which results in hypophosphatemia. It should be noted here that the Klotho-FGF receptor complex is expressed predominantly in the distal tubule, although the action occurs in the proximal tubule. The reason why is not clear but could possibly be explained by a proximal distal tubule feedback mechanism. The Klotho-FGF receptor complex is also expressed in the parathyroid gland. Animal studies have reported that FGF23 acts directly on the parathyroid gland to decrease PTH levels but the relation between FGF23 and PTH has yet to be clarified.

PTH-Vitamin D and Klotho-FGF 23 Bone-kidney Axis

Our current understanding of the PTH-Vitamin D axis in mineral metabolism suggests that PTH is primarily a 'calcemic' hormone. When calcium sensing receptors in the parathyroid glands sense that levels of serum calcium are low, they increase secretion of PTH, which targets kidney and bone. In the kidney, PTH increases calcium reabsorption, phosphate excretion, and $1\alpha, 25(\text{OH})_2\text{D}_3$ production (via stimulation of 1 alpha hydroxylase), which in turn increase calcium and phosphorus absorption from the intestine. In bone, PTH leads to calcium and phosphate efflux. Thus, the net effect is to restore calcium levels to normal.

The Klotho-FGF 23 axis is a primarily Vitamin D counter regulatory hormone with phosphaturic effects [17]. Serum FGF-23 concentrations increase following the administration of exogenous $1\alpha, 25(\text{OH})_2\text{D}_3$, and FGF-23 expression increases in bone cells following $1\alpha, 25(\text{OH})_2\text{D}_3$ treatment [14]. Increased 1,25-dihydroxyvitamin D_3 , with a resulting increase in calcium and phosphate, leads to the suppression of PTH and increased phosphate reabsorption in the proximal tubules. It has been shown that 1,25 dihydroxyvitamin D_3 (but not PTH, Calcium or Phosphate) stimulates FGF23 activity in the osteoblasts by acting on the vitamin D response element site that has been identified in the FGF 23 promoter [18]. Therefore, FGF23 is counter-regulatory to Vitamin D and helps to maintain phosphate balance.

Hyperphosphatemia and the Role of Phosphatonins

A serum phosphate concentration of more than 5mg/dl is considered hyperphosphatemia. In this state, the body induces a physiologically significant down-regulation of serum phosphate by reducing intestinal absorption of dietary phosphates and decreasing re-absorption of phosphate from glomerular filtrate. Unless there is a significant deterioration of renal function, the phosphate homeostasis is maintained by the action of PTH, Vitamin D_3 , and phosphatonins. The main underlying causes for hyperphosphatemia are decreased renal function, defective phosphatonin and other phosphaturic factors,

a large efflux of intracellular phosphates, and/or a dietary increase in phosphate [2].

Hyperphosphatemia accelerates renal tubulointerstitial disease, renal osteodystrophies and cardiovascular disease [19]. The latter is a major cause of death in patients with kidney disease. Adverse cardiovascular outcomes are associated with vascular calcification in patients with all stages of Chronic Kidney Disease (CKD). In vitro studies are quite consistent in showing that an elevated level of serum phosphorus can up regulate Cbfa1 (Runx 2), which is a protein that oversees the transformation of vascular smooth muscle cells into alkaline phosphatase and collagen-secreting osteoblasts. Elevated serum phosphate levels in the presence of normal or elevated serum calcium levels, leads to an increase in serum supersaturation, potentially overwhelming inhibitors of calcification and leading to the deposition of phosphorus and calcium on this extraosseous collagen matrix. A reduction in the Glomerular Filtration Rate (GFR), as seen in patients with CKD-associated renal insufficiency, is one of the major causes of hyperphosphatemia.

Sliem et al. [20] conducted a study in which the degree of hyperphosphatemia correlated positively with elevations of FGF23 in patients with CKD. In early CKD, the FGF23-klotho factor is elevated, which promotes urinary phosphate excretion, but as the disease advances, the phosphate loading may overcome this compensatory mechanism and a decreased glomerular filtration rate despite high FGF 23 levels will cause secondary hyperphosphatemia. In familial tumoral calcinosis, a mis-sense mutation in FGF23 production has been identified, and it renders the phosphatonin ineffective despite a normal GFR [13].

An efflux of intracellular phosphates is seen in tissue and cellular lysis. Conditions like crush injury, rhabdomyolysis, and tumor lysis syndrome cause hyperphosphatemia. A transcellular shift of phosphate is mediated via changes in pH as seen in metabolic acidosis and respiratory acidosis, resulting in hyperphosphatemia.

Causes of hyperphosphatemia

Reduced renal phosphate excretion: Patients with CKD develop hyperphosphatemia due to a reduction in the GFR, increased calcitriol (Vitamin D₃) levels and secondary hyperparathyroidism. Although the hyperphosphatemia is mild during early renal disease, it markedly worsens as kidney disease progresses and is an important marker of mortality in CKD patients [19].

Block et al. [21] studied a cohort of 40,538 hemodialysis patients and determined that 12% of the 10,015 deaths that occurred over the period of observation were independently associated with hyperphosphatemia. Initially, the rise in FGF23-klotho factor compensates for the reduced GFR by reducing the reabsorption of phosphate from the brush border by Na-Pi channels, thereby increasing the fractional filtration of phosphate. As CKD progresses and GFR deteriorates, however, this compensatory mechanism becomes ineffective, and the fractional excretion of phosphate decreases from 80% - 90% to as low as 15% [22]. A defect in the klotho gene leads to a steeper fall in the fractional excretion, as seen in Hyp mice and Familial Tumoral Calcinosis [13].

Topaz et al. [23] mapped the gene causing one form of tumoral calcinosis to 2q24-q31 and found homozygous or compound heterozygous mutations in GALNT3, which encodes a glycosyltransferase responsible for initiating mucin-type O-glycosylation. Interestingly, the concentrations of carboxyl-terminal FGF-23 were significantly elevated in those with tumoral calcinosis. These findings suggest

that defective post-translational modifications of FGF-23 could be responsible for the increased reabsorption of phosphates from the intestine and increased tubular reabsorption of phosphates from the glomerular filtrate, resulting in hyperphosphatemia.

Other causes of decreased phosphate excretion include parathyroid dysfunction as seen in pseudohypoparathyroidism, abnormal Plasma PTH, and hypoparathyroidism; calcium excess; pseudotumoral calcinosis; bisphosphate therapy; and metabolic alkalosis. Secondary hyperparathyroidism in CKD increases mortality by causing cardiovascular disease and renal osteodystrophy and accelerating renal tubulo-interstitial damage. Mineral homeostasis in CKD patients on dialysis is a very crucial part of treatment.

Increased Dietary phosphate: Phosphate is absorbed in the small intestine, and this process is mediated via Na-Pi IIB channels (intracellular processes) and the concentration gradient of luminal phosphate and serum phosphate (paracellular processes). Even though it is regulated by PTH and phosphatonins, it hardly reaches a point of saturation, and absorption of phosphate is proportional to the available dietary phosphate. A transient increase in absorbed phosphate is managed by the release of phosphaturic factor FGF23 and other phosphatonins in healthy individuals. PTH regulates a persistent increase in absorbed phosphate. Ritz et al. [24] stated that inorganic phosphate in food and food additives is effectively absorbed and can measurably elevate the serum phosphate concentration in patients with advanced CKD. Increased dietary intake of phosphate in the absence of any renal insufficiency rarely leads to hyperphosphatemia.

Efflux of intracellular phosphate: Levels of serum phosphate rise during enhanced catabolism, lactic acidosis, chronic respiratory alkalosis [25] and neoplastic diseases [26] such as leukemias and lymphomas due to an increased endogenous supply via an efflux of intracellular phosphate. Tumor lysis syndrome and enhanced catabolic states, which occur with hemolysis, rhabdomyolysis and malignant hyperthermia, cause hyperphosphatemia due to the redistribution of phosphate from the intracellular compartment to extracellular spaces. This transient rise, if left untreated, can itself cause renal tubulointerstitial damage and acute renal failure (ARF). Other factors can also affect this efflux of intracellular phosphorus including changes in pH (as seen in chronic respiratory alkalosis), increased dopamine levels and adrenergic activity [25,26] (Table 3).

Managing Hyperphosphatemia in CKD Patients

The first step is to limit phosphorus intake. However, phosphorus is a major component of protein, and any marked reductions in protein intake, which will lead to hypoalbuminemia, have clearly been shown to be detrimental to the survival of dialysis patients [27]. Thus, it is important that such patients maintain an adequate protein intake. Ingestion of large amounts of 1,25(OH)₂D₃ or its analogues should be avoided because all of these agents increase serum phosphorus levels by increasing intestinal phosphorus absorption [28] and possibly by promoting increased bone resorption [27].

It is important to limit elevated PTH levels in secondary hyperparathyroidism to prevent the efflux of phosphorus from bone. Cinacalcet reduces PTH while simultaneously lowering calcium and phosphorus levels whereas 1,25(OH)₂D₃ or its analogues also reduce PTH at the expense of an increase in serum phosphorus and calcium. A conventional hemodialysis treatment effectively removes a single day's worth of absorbed phosphorus [28]. Thus, the standard 3-d/wk dialysis treatment schedule is insufficient to remove phosphorus adequately

Function	Location of Expression	Type of Channel
Absorption of dietary phosphate	Villi in small intestine	Na-Pi Type IIb
Sodium phosphate reabsorption from the glomerular filtrate	Renal brush border membrane of Proximal Convoluted Tubule	Na-Pi type I, Na-Pi type IIa and IIc

Table 1: Sodium-Phosphate Co transport channels.

Type of Channel	Mainly expressed at	Function
Na-Pi type I	Renal brush border membrane of PCT	Sodium phosphate reabsorption from the glomerular filtrate
Na-Pi type II IIa	Renal brush border membrane of PCT	Sodium phosphate reabsorption from the glomerular filtrate
IIb	Villi in small intestine	absorption of dietary phosphate
IIc	Renal brush border membrane of PCT	Sodium phosphate reabsorption from the glomerular filtrate
Na-Pi type III	Renal sodium phosphate channels	Accounts for <1% encoded channels. May have housekeeping functions.

Table 2: Sodium-Phosphate Co transport channels & Functions.

Hyperphosphatemia	Hypophosphatemia
<p><i>Reduced renal phosphate excretion. eg</i></p> <ul style="list-style-type: none"> • Later stages of CKD • Variants of Tumoral Calcinosis • Biphosphate therapy • Metabolic alkalosis 	<p><i>Increased urinary excretion of phosphate. eg</i></p> <ul style="list-style-type: none"> • Tumor Induced osteomalacia • Autosomal Dominant hypophosphatemic rickets • X-linked hypophosphatemic rickets
<p><i>Efflux of intracellular phosphate. eg</i></p> <ul style="list-style-type: none"> • Tumor lysis syndrome • Catabolic states • Chronic respiratory alkalosis • Increased dopamine and adrenergic activity 	<p><i>Internal redistribution. eg</i></p> <ul style="list-style-type: none"> • Refeeding in malnourished patients • Increased intracellular pH • Hungry bone syndrome
<p><i>Increased Dietary phosphate</i></p>	<p><i>Decreased dietary intake of phosphate (Rare)</i></p>

Table 3: Mechanism of hyper- and hypo-phosphatemia.

on a weekly basis. A 4-hour hemodialysis session clears 34 mmol of phosphate (1054 mg), which is not sufficient to keep up with the typical phosphorus intake of 800 to 2000 mg per day (25.8–64.5 mmol). Phosphate efflux into the dialysate is greatest during the first hour of treatment, corresponding to the time during which serum phosphorus levels are highest. Phosphate efflux then falls off but remains at roughly half the initial value at the end of the treatment despite a stable serum phosphorus level. If the need for daily dialysis or lengthy three-times-per-week dialysis sessions becomes more prevalent, then phosphorus retention may be avoided. However, in most patients, it is necessary to bind intestinal phosphorus to lessen absorption and increase fecal excretion.

Phosphate Binders in Hyperphosphatemia

The ideal phosphorus binder should quickly and irreversibly bind large amounts of intestinal phosphorus, have no absorption or toxicity, and be inexpensive and easy to take [29]. None of the currently marketed phosphorus binders satisfies each of these parameters. Because of toxicity, aluminum is rarely used as a binder. The binders that are currently used are calcium-containing agents (calcium carbonate and calcium acetate) and non-calcium-containing agents (sevelamer hydrochloride, sevelamer carbonate, and lanthanum carbonate).

Suki et al. [30] conducted a multicenter, randomized, open-label

study to compare sevelamer hydrochloride with calcium-containing phosphate binders. They found that although all-cause mortality rates and cause-specific mortality rates were not different, mortality did seem to vary by age. In the patients who were older than 65 years, sevelamer hydrochloride had a significant effect in lowering the mortality rate whereas in younger patients, there was no difference. Block et al. [31] examined all-cause mortality in incident dialysis patients who were treated with sevelamer hydrochloride or calcium-containing phosphate binders. They found a reduction in mortality with sevelamer that persisted after multivariate adjustments.

Neither study was definitive—there was no difference in all-cause mortality in the Suki study and the number of patients in the Block study was small. However, no study has yet to show that the use of calcium-containing phosphate binders improves mortality better than non-calcium-containing binders.

Hypophosphatemia and the Role of FGF23

Hypophosphatemia is defined as a serum phosphate concentration less than 2.5 mg/dl and severe hypophosphatemia as less than 1mg/dl. Physiologically significant and symptomatic hypophosphatemia generally occurs when the serum phosphate concentration is below 1 mg/dl.

There are three major mechanisms of hypophosphatemia

Decreased dietary intake of phosphate. Inadequate dietary intake alone is rarely responsible for profound hypophosphatemia, especially since the body compensates with rapid renal adaptation and decreased urinary phosphate excretion in such situations. This mechanism is significant only in severe cases of malnutrition, where phosphate deprivation is severe and prolonged.

Redistribution of phosphate from the extracellular space into the intracellular space (internal redistribution) is the most common cause of hypophosphatemia in ICU patients. Carbohydrate refeeding in malnourished patients stimulates endogenous insulin release, which results in stimulation of glycolysis and formation of phosphorylated carbohydrate compounds in the liver and skeletal muscle. The source of this phosphate is the inorganic phosphate in the extracellular fluid, thus, serum phosphate concentrations will decrease rapidly. Respiratory alkalosis in patients with severe hyperventilation due to anxiety, pain, sepsis, or mechanical ventilation can lead to a decrease in the partial pressure of carbon dioxide and an increase in intracellular pH. The increase in intracellular pH stimulates phosphofructokinase and glycolysis. Alcoholic patients with respiratory alkalosis who are receiving concomitant glucose infusions are particularly vulnerable. The "hungry bone syndrome" is another cause of hypophosphatemia that can develop in patients who undergo parathyroidectomy after long-standing hyperparathyroidism [32]. This is due to a massive deposition of calcium and phosphorus in the osteopenic bone in the post-operative period.

Urinary excretion of phosphate by the kidney plays a major role in phosphate balance, and regulation of phosphate transport depends on phosphate concentration, parathyroid hormone and phosphatonins.

Primary or isolated renal phosphate wasting syndromes are characterized by normal parathyroid hormone levels and a low or normal calcitriol ($1,25(\text{OH})_2\text{D}_3$) levels. These include Tumor-Induced Osteomalacia (TIO) [10], X-Linked Hypophosphatemic rickets (XLH) [7], and Autosomal Dominant Hypophosphatemic Rickets (ADHR) [11].

Mechanism of FGF 23 Excess in Renal Phosphate Wasting Syndromes

X-linked hypophosphatemic rickets is characterized by hypophosphatemia, phosphaturia, slow growth, and rickets (osteomalacia). Mutations in the PHEX (Phosphate Regulating Endopeptidase on chromosome X) gene in bone tissue alter the degradation of FGF 23, causing increased circulating levels, which in turn cause phosphate wasting [7].

Autosomal dominant hypophosphatemic rickets produces signs and symptoms similar to those of XLH, although the symptoms vary according to the age of onset. If disease onset is early, clinical manifestations include phosphate wasting, rickets, and lower extremity deformities. After growth plate closure, patients generally present with bone pain, weakness, and fractures but no lower extremity deformities. Mutations in FGF 23, makes it resistant to proteolytic cleavage, so circulating levels of FGF23 increase, causing renal phosphate wasting [11].

Tumor-induced osteomalacia occurs in association with tumors that are typically benign, small and mesenchymal in origin. FGF 23 levels have been shown to decrease after removal of tumor. Thus, associated hypophosphatemia and osteomalacia are due to overproduction of

FGF23. Removing the tumor reduces circulating FGF23 levels and concomitantly, increases serum phosphate levels [10].

FGF 23 may play a role in post-transplantation hypophosphatemia. After transplant, hypophosphatemia may occur despite low PTH levels or even after high PTH levels normalize. Even in the setting of normal allograft function and hyperparathyroidism, hypophosphatemia with inappropriately normal allograft function and hyperparathyroidism, hypophosphatemia with inappropriately normal or low calcitriol levels are seen. Evenepoel et al. [33] studied 27 living donor transplant recipients and found that FGF 23 was independently associated with serum hypophosphatemia, urinary excretion of phosphate, and calcitriol levels in the early post transplant period; while PTH was not independently associated with any of these.

In a cross sectional study of 80 patients, Gutierrez et al. [34] found that increased FGF 23 levels were significantly associated with worsening renal function and decreased $1,25(\text{OH})_2\text{D}_3$ levels. While hyperphosphatemia was observed in advanced renal disease, levels of FGF 23 were significantly higher in the earlier stages of CKD. During early CKD, FGF23 may help maintain serum phosphate levels within the normal range (although the stimulus for increased FGF23 in the absence of hyperphosphatemia is unclear). This compensatory mechanism may be overwhelmed by severe renal failure and overt hyperphosphatemia in advanced CKD disease, despite markedly elevated FGF 23 and increased fractional excretion of phosphate. Thus, FGF 23 levels increase early in CKD before the development of serum mineral abnormalities.

Research also suggests that elevated FGF 23 levels can increase mortality in dialysis patients. In a prospective nested case-control study of 10,044 patients who were beginning hemodialysis, Gutierrez et al. [34] assessed the 1-year mortality risk based on FGF 23 levels. Increased FGF 23 levels were found to be independently associated with mortality, irrespective of serum phosphate levels [20,34]. This study was particularly significant in that even in patients considered to have normal serum phosphate levels; mortality was significantly higher in those patients who had higher FGF 23 levels. The role of FGF 23 as a potential biomarker that can potentially be used for management of phosphorus balance needs to be investigated in future studies.

Measurement of FGF 23

The full-length active hormone is cleaved at its RXXR motif into inactive amino acid and carboxy-terminal (c-terminal or cFGF 23 fragments by a proprotein convertase. The cFGF23 assays detect both intact FGF 23 (iFGF 23) and its c-terminal fragments. C-terminal fragments accumulate in patients with kidney disease. Gutierrez et al. [34] measured both cFGF 23 and iFGF 23 (which are specific for the intact molecule) in patients undergoing hemodialysis and found that the levels were significantly higher in cases having low survival rate after first year of initiation of treatment.

Currently, two iFGF 23 assay (Kainos; Immotopics) and one cFGF 23 assay (Immotopics) are commercially available, although they are not FDA approved for clinical use. Reference values established by the Mayo clinic medical laboratories for research use are as follows:

for age 3 months to 17 years, less than or equal to 230 RU/ml

for adults \geq 18yrs, less than or equal to 180 RU/ml

Frozen EDTA plasma specimens are preferred for analysis because serum specimens are less stable. The same specimen type (i.e serum or EDTA plasma) should be used for serial comparative measurements.

FGF 23 should always be interpreted in conjunction with serum phosphate levels. A normal FGF 23 (within reference range) should not necessarily discourage workup for oncogenic osteomalacia if this is strongly suspected, as other phosphatonins such as FRP 4 or MEPE may be responsible for hypophosphatemia in such patients.

Approach to the Patient with Hypophosphatemia

In a patient with hypophosphatemia, the clinical history helps to identify the possible mechanism that is involved. The normal renal response to hypophosphatemia is to increase reabsorption and decrease urinary excretion. Measurement of urinary phosphate excretion can be helpful in this regard, especially if the underlying mechanism is unclear. A 24-hour urine collection of phosphate or calculation for the fractional excretion of phosphate from a random urine specimen can be done. The fractional excretion of phosphate is calculated as:

$$FEPO_4 = [UPO_4 \times PCr \times 100] / [PPO_4 \times UCr]$$

U: urine concentration

P: plasma concentration

PO₄: phosphate

Cr: creatinine

With a normal renal response in the setting of hypophosphatemia, the daily phosphate excretion should be less than 100 mg and the fractional excretion of phosphate should be below 5% (normal is between 5% to 20%). If there is an appropriate renal response, then the probable mechanism of hypophosphatemia is either an internal redistribution (as occurs in refeeding syndrome, acute respiratory alkalosis, etc) or reduced intestinal absorption (as occurs in chronic diarrhea or chronic antacid intake). Inappropriately high phosphate excretion in the setting of hypophosphatemia is suggestive of renal wasting, which would include hyperparathyroidism or renal tubular defects (which may be isolated as in TIO, ADHR, XLR or generalized as in Fanconi syndrome). Laboratory investigations should be focused on confirming these diagnoses and include measurement of PTH, calcium, 25 (OH)₂D₃ and 1,25-(OH)₂D₃ levels.

If Fanconi syndrome is suspected, urinary amino acids and glucose should be measured. As discussed above, Fanconi syndrome is associated with aminoaciduria, glucosuria and hyperchloremic metabolic acidosis. If a generalized tubular dysfunction is found, this should prompt a search for the cause of Fanconi syndrome. Monoclonal proteins should be checked in serum and urine, as multiple myeloma is the most common cause of Fanconi syndrome in adults. A heavy metal screen should be performed, especially if there is a suggestive history of exposure, as this can also lead to proximal tubular dysfunction. Cadmium is of particular concern and is a byproduct of zinc refining in the battery industry. A blood level greater than 0.78 micrograms per liter or a urine level greater than 3.1 micrograms per gram of creatinine is diagnostic. Because it is associated with tubular proteinuria, it is helpful to measure urinary beta 2 microglobulin.

It should be noted that tubular proteinuria caused by cadmium is commonly seen after 25 years of exposure (it can be seen as early as 9 years after exposure). In comparison, lead exposure is not associated with beta 2 microglobulin, and Fanconi syndrome is unusual. In children, a diagnosis of Fanconi syndrome should prompt a search for inborn errors of metabolism.

A low level of calcitriol (1,25(OH)₂D₃) with hypophosphatemia is a clue to the presence of isolated renal phosphate wasting that can be

due to oncogenic osteomalacia (due to an underlying mesenchymal tumor) in adults. A low calcitriol level is seen, which is opposite of the expected stimulation of calcitriol production by hypophosphatemia in an attempt to raise the plasma phosphate concentration toward normal by increasing intestinal absorption and perhaps bone resorption. As discussed in earlier sections, measurement of FGF 23 would be helpful in such cases. However, it should be noted that this is not available clinically, and only specialized research labs currently have the ability to measure FGF 23.

Treatment

The focus of treatment should be directed at the underlying cause. Symptoms that are attributable to hypophosphatemia do not occur until levels are less than 2 mg/dl, and severe symptoms are not seen until levels are below 1mg/dl. Phosphate supplementation is needed in symptomatic patients and in those who have a renal tubular defect that is causing chronic renal phosphate wasting. Oral therapy is preferred with 2.5 to 3.5 grams given in divided doses. When intravenous therapy is necessary in the symptomatic patient, a maximum total dose of 30 mmol can be given over 6 hours in the setting of moderate hypophosphatemia. For more severe hypophosphatemia, a maximum total dose of 80 mmol can be given over 8 to 12 hours. It should be noted that potassium phosphate preparations can worsen hyperkalemia. For example, Neutra-phos has 7.1 mEq of potassium per packet whereas K-Phos neutral has only 1.1 mEq of potassium per packet.

Plasma phosphate concentrations should be closely monitored at least every 6 hours, especially when giving intravenous formulations. Vitamin D supplementation should be given in patients with vitamin D deficiency, with a recommended intake of at least 400 to 800 units per day.

A prospective study assessed whether chronic treatment with oral dipyridamole at a dose of 75 mg 4 times daily could decrease renal phosphate leak and increase serum phosphorus in patients with idiopathic low renal phosphate threshold. Serum phosphate significantly increased in 80% of patients within 3 months, with maximal values reached within 9 months. This improvement persisted after 12 months of treatment. PTH and calcium levels were unchanged, but the concentration of 1,25 (OH)₂D₃ was significantly decreased. After 2 years, treatment was discontinued in three patients, and serum phosphate decreased within 1 month after discontinuation. Further study is required to determine whether dipyridamole may be effective in this setting.

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