Crosstalk of fibroblast growth factor 23 and anemia-related factors during the development and progression of CKD (Review)

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Abstract. Fibroblast growth factor 23 (FGF23) plays an important role in the development of chronic kidney disease-mineral bone disorder (CKD-MBD). Abnormally elevated levels of 1,25-dihydroxyvitamin D cause osteocytes to secrete FGF23, which subsequently induces phosphaturia. Recent studies have reported that iron deficiency, erythropoietin (EPO) and hypoxia regulate the pathways responsible for FGF23 production. However, the molecular mechanisms underlying the interactions between FGF23 and anemia-related factors are not yet fully understood. The present review discusses the associations between FGF23, iron, EPO and hypoxia-inducible factors (HIFs), and their impact on FGF23 bioactivity, focusing on recent studies. Collectively, these findings propose interactions between FGF23 gene expression and anemia-related factors, including iron deficiency, EPO and HIFs. Taken together, these results suggest that FGF23 bioactivity is closely associated with the occurrence of CKD-related anemia and CKD-MBD.

Contents

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Abbreviations: FGF23, fibroblast growth factor 23; CKD-MBD, chronic kidney disease-mineral and bone disorder; 1,25[OH]2D, 1,25-dihydroxyvitamin D; EPO, erythropoietin; HIF-PHIs, hypoxia inducible factor-proline hydroxylase inhibitors; IV, intravenous; PHD, prolyl-hydroxylase

Key words: FGF23, iron, EPO, HIF, anemia, CKD-MBD

1. Introduction

Fibroblast growth factor 23 (FGF23) expression is elevated in early-stage chronic kidney disease (CKD), and continues to increase as the glomerular filtration rate decreases (1-4). FGF23 is a hormone derived from osteocytes that regulates the metabolism of phosphorus and 1,25-dihydroxyvitamin D (1,25[OH]2D). 1,25(OH)2D and high dietary phosphate intake upregulate FGF23 expression, resulting in increased renal excretion of phosphate and decreased synthesis of 1,25(OH)2D, which decreases FGF23 expression, thus completing a negative feedback loop (5). Recent studies have reported that iron deficiency (6-9) and erythropoietin (EPO) (10-13) also affect FGF23 production. In addition, a study on mice reported that applying hypoxia inducible factor-proline hydroxylase inhibitors (HIF-PHIs) increases the serum level of FGF23 (8). Several clinical studies on patients with kidney disease have demonstrated that increased FGF23 expression is associated with poor patient outcomes (14-17). High-dose EPO treatment also contributes to the high mortality and morbidity rates of patients diagnosed with CKD (18,19). Thus, it is important to determine the associations among FGF23, 1,25(OH)2D, EPO and HIFs to identify novel therapeutic targets for the treatment of CKD-mineral bone disorder (MBD) and renal anemia, and improve the prognosis of these patients. The present review aimed to discuss the associations among FGF23, iron, EPO and HIFs in CKD-MBD.

2. Clinical perspective

Iron deficiency, induced by overproduction of EPO or activation of HIFs, upregulates FGF23 expression and is associated with adverse events in patients with CKD (8). Thus, it is important to identify interventions that downregulate FGF23 expression to correct iron deficiency and improve patient outcomes.

A total of 79 single nucleotide polymorphisms (SNPs) in 29 genes are associated with CKD-MBD (20). Among these, five SNPs (rs1126616, rs35068180, rs1800247, rs4236 and rs2248359) are in proteins involved in mineral metabolism (osteocalcin, osteopontin, Gla protein matrix metalloprotease 3, and 24 hydroxylase), which may be responsible for upregulating FGF23 expression (20). A Previous study has demonstrated that FGF23 expression closely parallels plasma EPO expression, either from administration of exogenous

EPO or increased production of endogenous EPO, induced by HIF-PHIs (12).

The present review compares the induction of FGF23 expression by EPO administration and HIF-PHIs, and discusses optimal therapeutic regimens for CKD-related anemia and MBD. EPO is associated with an increase in the inactive form of FGS23, C-terminal (c)FGF23, which can result in adverse events (21). Thus, the biological activity of cFGF23 requires further investigation.

3. FGF23 and iron deficiency

Regulation of FGF23 by iron deficiency. Iron functions as a cofactor in several enzymatic reactions and a critical component of hemoglobin, which is required for normal oxygen transport (22). Several factors can cause iron deficiency and anemia, including pregnancy (23), poor diet (24-26), inflammation (27,28), iron malabsorption (29,30) and CKD (31,32). A study on patients with CKD reported an association between low iron levels and high cFGF23 expression levels (33). Recently, Eisenga *et al* (34) confirmed that iron deficiency is associated with high serum FGF23 levels in patients with CKD and kidney transplant recipients (9,34). Molecular studies have reported that iron deficiency upregulates furin by stabilizing HIF1-α, which subsequently cleaves FGF23 into cFGF23 fragments (6,35,36). Furthermore, Eisenga *et al* (37) demonstrated that increased FGF23 levels mediate the association between iron deficiency and mortality. Iron deficiency, as a crucial determinant of FGF23 expression, can also occur during blood loss from trauma, surgery and bowel dysfunction (38,39). Rabadi *et al* (11) observed that acute bleeding elevated cFGF23 expression in healthy mice, and that the number of transfusions was positively associated with cFGF23 expression in patients in intensive care. It has also been demonstrated that GalNac-transferase 3 bone marrow mRNA expression decreases in mice following acute blood loss, which protects intact (i)FGF23 from proteolysis by furin, and thus enhances FGF23 cleavage (40). These findings regarding the regulation of FGF23 via iron deficiency are consistent with previous studies (35,36). However, a recent study reported that iFGF23 expression has a significantly negative association with serum iron parameters in elderly men (41). Presumably, certain age-related factors may affect the cleavage of FGF23 in individuals with iron deficiency.

Previous studies have demonstrated that iron deficiency increases cFGF23 expression, and that iFGF23 expression may remain normal due to FGF23 cleavage (42). However, the factors that affect FGF23 cleavage remain unclear. In particular, the functions of cleaved fragments in patients with CKD, such as cFGF23, remain largely unknown.

Impact of iron supplementation on FGF23. The physiological mechanisms underlying the association between increased FGF23 expression and mortality are presented in Fig. 1. FGF23 can directly induce left ventricular hypertrophy and eventually lead to heart failure, during which time oxidative stress enhances, the level of nitric oxide decreases and vasodilatation is impaired. FGF23 can increase the extent of vascular calcification and risk of cardiovascular disease. Vascular calcification may promote dysregulation of bone mineral metabolism, aggravate anemia and inflammatory response, and contribute to the progression of CKD. In addition, FGF23 increases the likelihood of cancer and mortality risk, and these factors may interact with one another. For example, inflammation may affect vasodilatation by promoting vascular calcification directly or indirectly through bone and mineral metabolism disorder. Anemia aggravates heart failure by increasing left ventricular hypertrophy, and aggravates mineral metabolism disorder. Conversely, bone mineral metabolism disorder and inflammation further promote the occurrence of anemia (43). Given the effect of elevated FGF23 expression on mortality and other pathophysiological outcomes (44-51), it is important to understand the pathways and molecular mechanisms underlying elevated FGF23 expression. As a major determinant of FGF23, iron deficiency can be easily modified to decrease FGF23 expression. Thus, the potential therapeutic benefits of iron supplementation should be considered. A previous study reported the effect of iron supplementation on elevated iFGF23 expression in two patients with osteomalacia (52). Currently, two major iron formulations are used as supplements, oral iron and intravenous (IV) iron. Patients with early-stage (non-dialysis) CKD often receive oral iron supplementation as treatment for iron deficiency and mild anemia. Clinicians administer IV iron to circumvent gastrointestinal intolerance and improve treatment efficacy, particularly in patients with late-stage CKD who are receiving hemodialysis (53).

A prospective randomized study assessed the use of oral iron and IV iron for 10 weeks as treatment for patients with CKD who were receiving hemodialysis and had iron-deficiency anemia. Serum cFGF23 levels decreased in both groups, while serum iFGF23 levels increased in the IV iron group (54). These findings suggest that oral iron is superior to IV iron in preventing high iFGF23 expression levels. However, the majority of patients with CKD also received EPO or EPO-stimulating agents, the effects of which were not compared between both groups. Thus, whether EPO administration affects the iFGF23/cFGF23 ratio remains unclear.

Hyperphosphatemia, due to decreased phosphate secretion, is significantly associated with CKD progression. Thus, researchers have developed several novel phosphate binders as treatment (55). Sucroferric oxyhydroxide effectively decreases serum phosphorus levels (56) and simultaneously improves iron parameters (57). Notably, serum FGF23 levels markedly decrease following treatment with sucroferric oxyhydroxide in patients with CKD undergoing hemodialysis (58). Ferric citrate, another iron-based phosphate binder, efficiently decreases serum phosphorus levels (59) and notably improves iron parameters (60), while simultaneously decreasing serum FGF23 levels in patients with CKD undergoing hemodialysis (61). Thus, concurrent reduction of serum phosphorus levels and correction of iron deficiency may be used to effectively decrease iFGF23 expression.

Taken together, these findings suggest that elevated serum FGF23 levels contribute to high mortality in patients with CKD. The correction of iron deficiency, a major determinant of elevated FGF23 expression, can reverse high FGF23

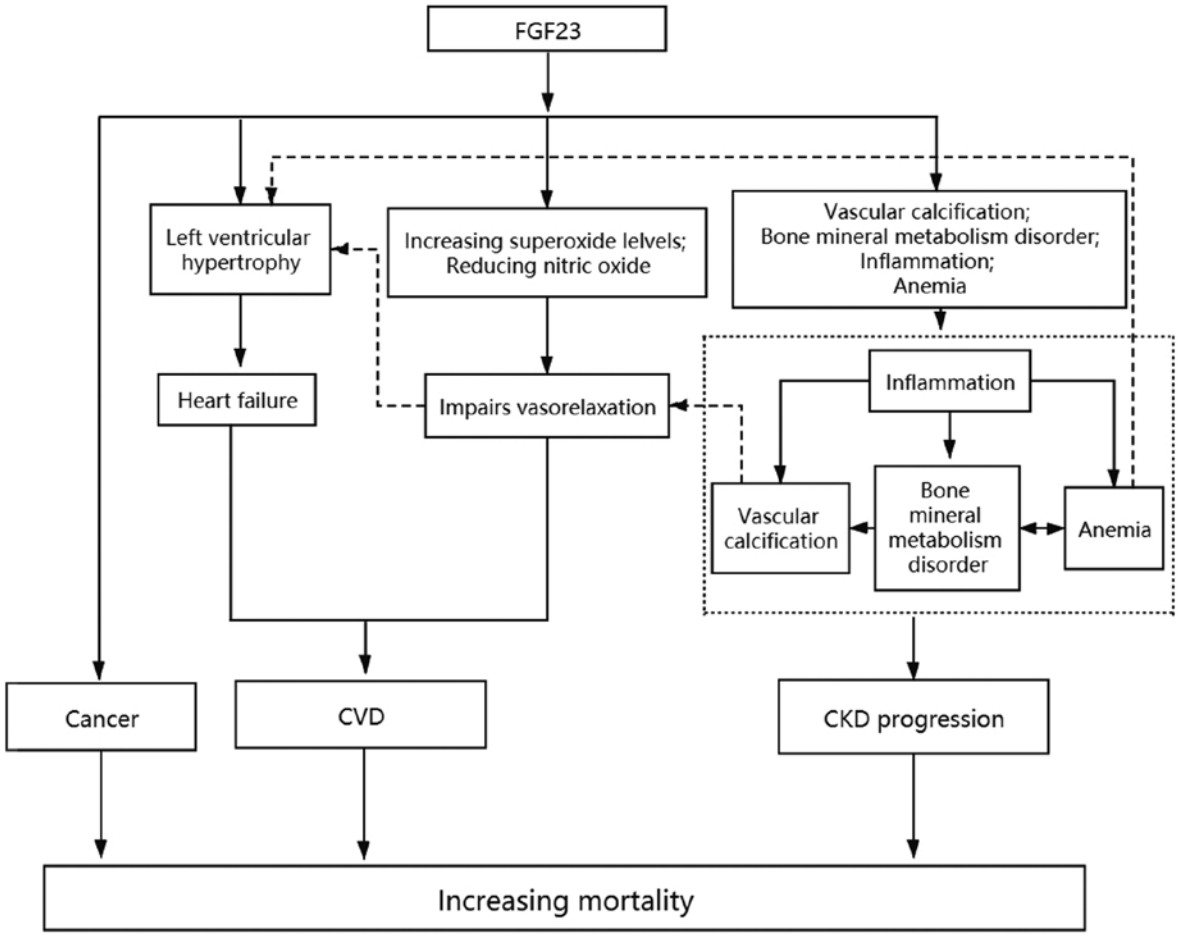

Figure 1. Physiological mechanisms underlying the association between increased FGF23 expression and high mortality risk. Solid lines indicate direct effects, while dashed lines indicate indirect effects. FGF23, fibroblast growth factor 23; CVD, cardiovascular disease; CKD, chronic kidney disease.

expression levels. Clinical studies have indicated that oral iron is superior to IV iron. Iron-based phosphate treatment, which simultaneously decreases phosphorus levels and corrects iron deficiency, may be used to decrease serum FGF23 levels and improve the long-term outcomes of these patients.

4. FGF23 and EPO

FGF23 and recombinant (rh)EPO administration. EPO is a hematopoietic hormone that is primarily produced by the kidneys as a physiological response to iron deficiency (62). Iron deficiency and anemia are co-morbidities in CKD (63) that promote renal function decline (64). There are several causes of iron deficiency in CKD, but reduced EPO synthesis is the main factor (65). Thus, clinicians often administer rhEPO to correct iron deficiency and anemia in these patients (66). However, the effect of rhEPO administration on serum FGF23 levels remains unclear. Previous studies have reported that acute injections of rhEPO significantly increase the expression levels of cFGF23 (11) and iFGF23 (10,67) in mice. Conversely, it has been demonstrated that rhEPO injections only increase cFGF23 expression in mice with CKD (68). Notably, these observed effects were independent of iron status. Studies on patients with chronic heart failure and CKD have reported that exogenous EPO injections markedly increase cFGF23 expression (69,70). The potential reasons for these differences may be attributed to the differences in renal functions of patients and/or the dose of rhEPO used. A previous study demonstrated that high cFGF23 expression competes with iFGF23 by binding to the FGF receptor, thereby decreasing iFGF23 expression (71). Another study on adult rats reported that *in vitro* treatment with cFGF23 increases the surface area of ventricular myocytes (72). Thus, it is important to identify which specific fragments of FGF23 are responsible for its effect on mortality. Prospective studies are required to assess the biological activity of different FGF23 fragments, and the effects of rhEPO replacement treatment on FGF23 expression in patients with CKD.

FGF23 and HIFs. HIFs are proteins that regulate transcription in response to low cellular oxygen levels. HIF prolyl-hydroxylase (PHD) regulates HIFs in an oxygen-dependent manner (73). HIFs are heterodimers composed of an α subunit and β subunit; HIF-α is oxygen-sensitive and stabilized due to decreased PHD-dependent hydroxylation during hypoxia (74-76). HIF-PHI stabilizes HIFs by stimulating hypoxia, thereby activating HIF signaling, inducing the transcription of endogenous EPO (77), promoting iron uptake and availability (78), and further influencing FGF23 expression (8,79,80). The HIF-PH inhibitor, roxadustat, has

been used as an oral drug for the treatment of anemia (81). This drug increases FGF23 production, suggesting that HIF-PHI may affect FGF23 expression and cleavage by inducing the transcription of endogenous EPO (80). Generally, FGF23 produced by osteocytes is affected by the level of phosphate, parathyroid hormone and 1,25(OH)2D (82). However, a recent study reported that other factors may regulate FGF23 production and cleavage, including iron, HIF and EPO signaling (83). Recent data have demonstrated that FGF23 expression may be directly regulated by HIF signaling in osteogenic cells, or indirectly regulated by EPO (12,82). Another study reported that treatment with HIF-PHI increases FGF23 expression (8). Examinations of the underlying molecular mechanism have demonstrated that inflammation or iron deficiency induce HIF-1α (8), and that binding of HIF-1α to the FGF23 promoter increases its synthesis in osteogenic cells (84). In addition, HIF-1α may also indirectly increase FGF23 expression by inducing EPO, thus promoting FGF23 transcription and cleavage (10-12,68,85). It has been reported that interleukin-6 and tumor necrosis factor-α levels are induced during CKD, indicating a general microinflammatory state in patients with CKD (86). FGF23 bioactivity and anti-inflammatory cytokine expression are abnormal in patients with CKD, which may be associated with the widespread microinflammatory state (87).

Low 1,25(OH)2D expression is associated with nutritional deficiency or endogenous resistance of EPO in patients with CKD, and often contributes to anemia (88). However, these associations are not affected by inflammatory status or secondary hyperparathyroidism (89). 1,25(OH)2D3 upregulates HIF in PMA-differentiated U937 cells, which is inhibited by rapamycin, suggesting that mTOR signaling is also involved in this process (90).

Administrating an anti-EPO antibody abrogates the effect of EPO on upregulation of FGF23 (91). This confirms that HIF-PHI increases FGF23 expression by induction of EPO (8). Notably, the majority of FGF23 induced via endogenous EPO following treatment with HIF-PHI is cFGF23, instead of iFGF23 (8,12). However, the pathological significance of increased expression levels of cFGF23 fragments remains unclear. Normal plasma iFGF23 levels are maintained in spite of increased FGF23 expression due to increased cleavage of iFGF23 (35). HIF-PHI efficiently induces erythropoiesis due to upregulation of endogenous EPO, but because the EPO level remains near the normal physiological range this treatment is superior to traditional rhEPO administration for treatment of renal anemia (92). Thus, clinicians should consider the use of HIF-PHI as a novel treatment strategy for renal anemia. A recent study reported that iFGF23 expression is not associated with rhEPO dose in hemodialysis patients (93). However, some unrecognized confounders may affect the measurement of FGF23 during CKD, which require further investigation in future studies.

5. Conclusions

In conclusion, iron deficiency, mediated by HIF1α and EPO, independently increases FGF23 expression and promotes the cleavage of FGF23, which is dependent on age and renal function. HIF-PHI induces the transcription of endogenous EPO, although EPO remains within its normal physiological range, and it also affects FGF23 expression and cleavage (12). Further studies are required to determine how endogenous or exogenous EPO increases FGF23 expression and affects FGF23 cleavage. Generally, endogenous EPO levels are elevated during early-stage CKD (94), exogenous EPO is typically administered to patients with late-stage CKD due to the presence of renal anemia, and FGF23 cleavage may decrease as renal function declines (95,96). iFGF23 is considered the bioactive form, and high cFGF23 expression is associated with poor prognosis in patients with CKD (17,69). Future studies are required to determine the biological activities of the C-terminal fragments of FGF23. The compensatory increase in FGF23 expression during the early stages of CKD may enhance urinary phosphate excretion, which may be beneficial to patients with CKD. The induction of FGF23 expression by EPO administration or HIF-PHIs may provide therapeutic regimens for the treatment of patients with CKD. In addition, the potential therapeutic benefits of iron supplementation should be considered, particularly in early-stage CKD with iron deficiency and mild anemia. However, the continuous increase in FGF23 expression during late stages of CKD may aggravate CKD-MBD and promote CKD-related anemia (97). A recent study suggested that blockade of FGF23 signaling prevents renal anemia in a murine model of CKD (21). Future studies must consider the complex molecular and physiological interactions that occur during renal anemia and CKD-MBD to develop novel therapeutic interventions.

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Authors' contributions

JBZ designed the present study and performed the literature review. RZ drafted the initial manuscript. SYW and FY analyzed the physiopathological mechanisms . SM, XL and CK contributed to the interpretation of the molecular mechanisms. All authors have read and approved the final manuscript. Data sharing is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Isakova T, Wahl P, Vargas GS, Gutiérrez OM, Scialla J, Xie H, Appleby D, Nessel L, Bellovich K, Chen J, *et al*: Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. Kidney Int 79: 1370-1378, 2011.
2. Larsson T, Nisbeth U, Ljunggren O, Juppner H and Jonsson KB: Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. Kidney Int 64: 2272-2279, 2003.
3. Gutierrez O, Isakova T, Rhee E, Shah A, Holmes J, Collerone G, Juppner H and Wolf M: Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. J Am Soc Nephrol 16: 2205-2215, 2005.
4. Portale AA, Wolf M, Jüppner H, Messinger S, Kumar J, Wesseling-Perry K, Schwartz GJ, Furth SL, Warady BA and Salusky IB: Disordered FGF23 and mineral metabolism in children with CKD. Clin J Am Soc Nephrol 9: 344-353, 2014.
5. Han X and Quarles LD: Multiple faces of fibroblast growth factor-23. Curr Opin Nephrol Hypertens 25: 333-342, 2016.
6. Farrow EG, Yu X, Summers LJ, Davis SI, Fleet JC, Allen MR, Robling AG, Stayrook KR, Jideonwo V, Magers MJ, *et al*: Iron deficiency drives an autosomal dominant hypophosphatemic rickets (ADHR) phenotype in fibroblast growth factor-23 (Fgf23) knock-in mice. Proc Natl Acad Sci USA 108: E1146-E1155, 2011.
7. Clinkenbeard EL, Farrow EG, Summers LJ, Cass TA, Roberts JL, Bayt CA, Lahm T, Albrecht M, Allen MR, Peacock M and White KE: Neonatal iron deficiency causes abnormal phosphate metabolism by elevating FGF23 in normal and ADHR mice. J Bone Miner Res 29: 361-369, 2014.
8. David V, Martin A, Isakova T, Spaulding C, Qi L, Ramirez V, Zumbrennen-Bullough KB, Sun CC, Lin HY, Babitt JL and Wolf M: Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. Kidney Int 89: 135-146, 2016.
9. Hanudel MR, Chua K, Rappaport M, Gabayan V, Valore E, Goltzman D, Ganz T, Nemeth E and Salusky IB: Effects of dietary iron intake and chronic kidney disease on fibroblast growth factor 23 metabolism in wild-type and hepcidin knockout mice. Am J Physiol Renal Physiol 311: F1369-F1377, 2016.
10. Clinkenbeard EL, Hanudel MR, Stayrook KR, Appaiah HN, Farrow EG, Cass TA, Summers LJ, Ip CS, Hum JM, Thomas JC, *et al*: Erythropoietin stimulates murine and human fibroblast growth factor-23, revealing novel roles for bone and bone marrow. Haematologica 102: e427-e430, 2017.
11. Rabadi S, Udo I, Leaf DE, Waikar SS and Christov M: Acute blood loss stimulates fibroblast growth factor 23 production. Am J Physiol Renal Physiol 314: F132-F139, 2018.
12. Flamme I, Ellinghaus P, Urrego D and Krüger T: FGF23 expression in rodents is directly induced via erythropoietin after inhibition of hypoxia inducible factor proline hydroxylase. PLoS One 12: e0186979, 2017.
13. Toro L, Barrientos V, León P, Rojas M, Gonzalez M, González-Ibáñez A, Illanes S, Sugikawa K, Abarzua N, Bascunan C, *et al*: Erythropoietin induces bone marrow and plasma fibroblast growth factor 23 during acute kidney injury. Kidney Int 93: 1131-1141, 2018.
14. Souma N, Isakova T, Lipiszko D, Sacco RL, Elkind MS, DeRosa JT, Silverberg SJ, Mendez AJ, Dong C, Wright CB, *et al*: Fibroblast growth factor 23 and cause-specific mortality in the general population: The Northern Manhattan Study. J Clin Endocrinol Metab 101: 3779-3786, 2016.
15. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Jüppner H and Wolf M: Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 359: 584-592, 2008.
16. Baia LC, Humalda JK, Vervloet MG, Navis G, Bakker SJ and de Borst MH; NIGRAM Consortium: Fibroblast growth factor 23 and cardiovascular mortality after kidney transplantation. Clin J Am Soc Nephrol 8: 1968-1978, 2013.
17. Isakova T, Xie H, Yang W, Xie D, Anderson AH, Scialla J, Wahl P, Gutierrez OM, Steigerwalt S, He J, *et al*: Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. Jama 305: 2432-2439, 2011.
18. Szczech LA, Barnhart HX, Inrig JK, Reddan DN, Sapp S, Califf RM, Patel UD and Singh AK: Secondary analysis of the CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. Kidney Int 74: 791-798, 2008.
19. Lestz RM, Fivush BA and Atkinson MA: Association of higher erythropoiesis stimulating agent dose and mortality in children on dialysis. Pediatr Nephrol 29: 2021-2028, 2014.
20. Valls J, Cambray S, Pérez-Guallar C, Bozic M, Bermúdez-López M, Fernández E, Betriu À, Rodríguez I and Valdivielso JM: Association of candidate gene polymorphisms with chronic kidney disease: Results of a case-control analysis in the Nefrona Cohort. Front Genet 10: 118, 2019.
21. Agoro R, Montagna A, Goetz R, Aligbe O, Singh G, Coe LM, Mohammadi M, Rivella S and Sitara D: Inhibition of fibroblast growth factor 23 (FGF23) signaling rescues renal anemia. FASEB J 32: 3752-3764, 2018.
22. Geissler C and Singh M: Iron, meat and health. Nutrients 3: 283-316, 2011.
23. Brannon PM and Taylor CL: Iron supplementation during pregnancy and infancy: Uncertainties and implications for research and policy. Nutrients 9: 1327, 2017.
24. Müller O and Krawinkel M: Malnutrition and health in developing countries. CMAJ 173: 279-286, 2005.
25. Skalicky A, Meyers AF, Adams WG, Yang Z, Cook JT and Frank DA: Child food insecurity and iron deficiency anemia in low-income infants and toddlers in the United States. Matern Child Health J 10: 177-185, 2006.
26. Díaz-Castro J, López-Frías MR, Campos MS, López-Frías M, Alférez MJ, Nestares T, Ojeda ML and López-Aliaga I: Severe nutritional iron-deficiency anaemia has a negative effect on some bone turnover biomarkers in rats. Eur J Nutr 51: 241-247, 2012.
27. Cartwright GE, Lauritsen MA, Humphreys S, Jones PJ, Merrill IM and Wintrobe MM: The anemia associated with chronic infection. Science 103: 72-73, 1946.
28. Cartwright GE, Lauritsen MA, Jones PJ, Merrill IM and Wintrobe MM: The anemia of infection; hypoferremia, hypercupremia, and alterations in porphyrin metabolism in patients. J Clin Invest 25: 65-80, 1946.
29. Qamar K, Saboor M, Qudsia F, Khosa SM, Moinuddin and Usman M: Malabsorption of iron as a cause of iron deficiency anemia in postmenopausal women. Pak J Med Sci 31: 304-308, 2015.
30. Filmann N, Rey J, Schneeweiss S, Ardizzone S, Bager P, Bergamaschi G, Koutroubakis I, Lindgren S, Morena Fde L, Moum B, *et al*: Prevalence of anemia in inflammatory bowel diseases in european countries: A systematic review and individual patient data meta-analysis. Inflamm Bowel Dis 20: 936-945, 2014.
31. Gotloib L, Silverberg D, Fudin R and Shostak A: Iron deficiency is a common cause of anemia in chronic kidney disease and can often be corrected with intravenous iron. J Nephrol 19: 161-167, 2006.
32. Lankhorst CE and Wish JB: Anemia in renal disease: Diagnosis and management. Blood Rev 24: 39-47, 2010.
33. Mehta R, Cai X, Hodakowski A, Lee J, Leonard M, Ricardo A, Chen J, Hamm L, Sondheimer J, Dobre M, *et al*: Fibroblast growth factor 23 and anemia in the chronic renal insufficiency cohort study. Clin J Am Soc Nephrol 12: 1795-1803, 2017.
34. Eisenga MF, van Londen M, Leaf DE, Nolte IM, Navis G, Bakker SJL, de Borst MH and Gaillard C: C-terminal fibroblast growth factor 23, iron deficiency, and mortality in renal transplant recipients. J Am Soc Nephrol 28: 3639-3646, 2017.
35. Wolf M and White KE: Coupling fibroblast growth factor 23 production and cleavage: Iron deficiency, rickets, and kidney disease. Curr Opin Nephrol Hypertens 23: 411-419, 2014.
36. McMahon S, Grondin F, McDonald PP, Richard DE and Dubois CM: Hypoxia-enhanced expression of the proprotein convertase furin is mediated by hypoxia-inducible factor-1: Impact on the bioactivation of proproteins. J Biol Chem 280: 6561-6569, 2005.
37. Eisenga MF, De Jong MA, Van der Meer P, Leaf DE, Huls G, Nolte IM, Gaillard C, Bakker SJL and De Borst MH: Iron deficiency, elevated erythropoietin, fibroblast growth factor 23, and mortality in the general population of the Netherlands: A cohort study. PLoS Med 16: e1002818, 2019.
38. Vieth JT and Lane DR: Anemia. Emerg Med Clin North Am 32: 613-628, 2014.
39. Bryan LJ and Zakai NA: Why is my patient anemic? Hematol Oncol Clin North Am 26: 205-230, vii, 2012.
40. Kido S, Fujihara M, Nomura K, Sasaki S, Mukai R, Ohnishi R, Kaneko I, Segawa H, Tatsumi S, Izumi H, *et al*: Molecular mechanisms of cadmium-induced fibroblast growth factor 23 upregulation in osteoblast-like cells. Toxicol Sci 139: 301-316, 2014.

41. Lewerin C, Ljunggren O, Nilsson-Ehle H, Karlsson MK, Herlitz H, Lorentzon M, Ohlsson C and Mellstrom D: Low serum iron is associated with high serum intact FGF23 in elderly men: The Swedish MrOS study. Bone 98: 1-8, 2017.
42. David V, Francis C and Babitt JL: Ironing out the cross talk between FGF23 and inflammation. Am J Physiol Renal Physiol 312: F1-F8, 2017.
43. Ärnlöv J, Carlsson AC, Sundström J, Ingelsson E, Larsson A, Lind L and Larsson TE: Serum FGF23 and risk of cardiovascular events in relation to mineral metabolism and cardiovascular pathology. Clin J Am Soc Nephrol 8: 781-786, 2013.
44. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, Gutierrez OM, Aguillon-Prada R, Lincoln J, Hare JM, *et al*: FGF23 induces left ventricular hypertrophy. J Clin Invest 121: 4393-4408, 2011.
45. Silswal N, Touchberry CD, Daniel DR, McCarthy DL, Zhang S, Andresen J, Stubbs JR and Wacker MJ: FGF23 directly impairs endothelium-dependent vasorelaxation by increasing superoxide levels and reducing nitric oxide bioavailability. Am J Physiol Endocrinol Metab 307: E426-E436, 2014.
46. Smith ER, Tan SJ, Holt SG and Hewitson TD: FGF23 is synthesised locally by renal tubules and activates injury-primed fibroblasts. Sci Rep 7: 3345, 2017.
47. Singh S, Grabner A, Yanucil C, Schramm K, Czaya B, Krick S, Czaja MJ, Bartz R, Abraham R, Di Marco GS, *et al*: Fibroblast growth factor 23 directly targets hepatocytes to promote inflammation in chronic kidney disease. Kidney Int 90: 985-996, 2016.
48. Rossaint J, Oehmichen J, Van Aken H, Reuter S, Pavenstadt HJ, Meersch M, Unruh M and Zarbock A: FGF23 signaling impairs neutrophil recruitment and host defense during CKD. J Clin Invest 126: 962-974, 2016.
49. Corn PG, Wang F, McKeehan WL and Navone N: Targeting fibroblast growth factor pathways in prostate cancer. Clin Cancer Res 19: 5856-5866, 2013.
50. Turner N and Grose R: Fibroblast growth factor signalling: From development to cancer. Nat Rev Cancer 10: 116-129, 2010.
51. Feng S, Wang J, Zhang Y, Creighton CJ and Ittmann M: FGF23 promotes prostate cancer progression. Oncotarget 6: 17291-17301, 2015.
52. Okada M, Imamura K, Fuchigami T, Omae T, Iida M, Nanishi F, Murakami M, Ohgushi H, Yao T, Fujita K and Ogawa K: 2 cases of nonspecific multiple ulcers of the small intestine associated with osteomalacia caused by long-term intravenous administration of saccharated ferric oxide. Nihon Naika Gakkai Zasshi 71: 1566-1572, 1982 (In Japanese).
53. Shepshelovich D, Rozen-Zvi B, Avni T, Gafter U and Gafter-Gvili A: Intravenous versus oral iron supplementation for the treatment of anemia in CKD: An updated systematic review and meta-analysis. Am J Kidney Dis 68: 677-690, 2016.
54. Fukao W, Hasuike Y, Yamakawa T, Toyoda K, Aichi M, Masachika S, Kantou M, Takahishi SI, Iwasaki T, Yahiro M, *et al*: Oral versus intravenous iron supplementation for the treatment of iron deficiency anemia in patients on maintenance hemodialysis-effect on fibroblast growth factor-23 metabolism. J Ren Nutr 28: 270-277, 2018.
55. Taniguchi K and Kakuta H: Bixalomer, a novel phosphate binder with a small swelling index, improves hyperphosphatemia in chronic kidney disease rat. Eur J Pharmacol 766: 129-134, 2015.
56. Koiwa F, Yokoyama K, Fukagawa M, Terao A and Akizawa T: Efficacy and safety of sucroferric oxyhydroxide compared with sevelamer hydrochloride in Japanese haemodialysis patients with hyperphosphataemia: A randomized, open-label, multicentre, 12-week phase III study. Nephrology (Carlton) 22: 293-300, 2017.
57. Covic AC, Floege J, Ketteler M, Sprague SM, Lisk L, Rakov V and Rastogi A: Iron-related parameters in dialysis patients treated with sucroferric oxyhydroxide. Nephrol Dial Transplant 32: 1330-1338, 2017.
58. Shima H, Miya K, Okada K, Minakuchi J and Kawashima S: Sucroferric oxyhydroxide decreases serum phosphorus level and fibroblast growth factor 23 and improves renal anemia in hemodialysis patients. BMC Res Notes 11: 363, 2018.
59. Yang WC, Yang CS, Hou CC, Wu TH, Young EW and Hsu CH: An open-label, crossover study of a new phosphate-binding agent in haemodialysis patients: Ferric citrate. Nephrol Dial Transplant 17: 265-270, 2002.
60. Lee CT, Wu IW, Chiang SS, Peng YS, Shu KH, Wu MJ and Wu MS: Effect of oral ferric citrate on serum phosphorus in hemodialysis patients: Multicenter, randomized, double-blind, placebo-controlled study. J Nephrol 28: 105-113, 2015.
61. Maruyama N, Otsuki T, Yoshida Y, Nagura C, Kitai M, Shibahara N, Tomita H, Maruyama T and Abe M: Ferric citrate decreases fibroblast growth factor 23 and improves erythropoietin responsiveness in hemodialysis patients. Am J Nephrol 47: 406-414, 2018.
62. National Clinical Guideline Centre (UK): Anaemia Management in Chronic Kidney Disease: Partial Update 2015 (Internet). London: Royal College of Physicians (UK), Jun 2015. https://www.ncbi.nlm.nih.gov/books/NBK299242/.
63. Babitt JL and Lin HY: Mechanisms of anemia in CKD. J Am Soc Nephrol 23: 1631-1634, 2012.
64. Hinata A, Iijima M, Nakano Y, Sakamoto T and Tomita M: Chemical characterization of rabbit alpha 2-macroglobulin. Chem Pharm Bull (Tokyo) 35: 271-276, 1987.
65. Landau D, London L, Bandach I and Segev Y: The hypoxia inducible factor/erythropoietin (EPO)/EPO receptor pathway is disturbed in a rat model of chronic kidney disease related anemia. PLoS One 13: e0196684, 2018.
66. Thomas S and Rampersad, M: Anaemia in diabetes. Acta diabetologica 41 (Suppl 1): S13-S17, 2004.
67. Daryadel A, Bettoni C, Haider T, Imenez Silva PH, Schnitzbauer U, Pastor-Arroyo EM, Wenger RH, Gassmann M and Wagner CA: Erythropoietin stimulates fibroblast growth factor 23 (FGF23) in mice and men. Pflugers Arch 470: 1569-1582, 2018.
68. Hanudel MR, Eisenga MF, Rappaport M, Chua K, Qiao B, Jung G, Gabayan V, Gales B, Ramos G, de Jong MA, *et al*: Effects of erythropoietin on fibroblast growth factor 23 in mice and humans. Nephrol Dial Transplant 34: 2057-2065, 2019.
69. Eisenga MF, Emans ME, van der Putten K, Cramer MJ, Diepenbroek A, Velthuis BK, Doevendans PA, Verhaar MC, Joles JA, Bakker SJL, *et al*: Epoetin Beta and C-terminal fibroblast growth factor 23 in patients with chronic heart failure and chronic kidney disease. J Am Heart Assoc 8: e011130, 2019.
70. van der Putten K, Braam B, Jie KE and Gaillard CA: Mechanisms of disease: Erythropoietin resistance in patients with both heart and kidney failure. Nat Clin Pract Nephrol 4: 47-57, 2008.
71. Goetz R, Nakada Y, Hu MC, Kurosu H, Wang L, Nakatani T, Shi M, Eliseenkova AV, Razzaque MS, Moe OW, *et al*: Isolated C-terminal tail of FGF23 alleviates hypophosphatemia by inhibiting FGF23-FGFR-Klotho complex formation. Proc Natl Acad Sci USA 107: 407-412, 2010.
72. Courbebaisse M, Mehel H, Petit-Hoang C, Ribeil JA, Sabbah L, Tuloup-Minguez V, Bergerat D, Arlet JB, Stanislas A, Souberbielle JC, *et al*: Carboxy-terminal fragment of fibroblast growth factor 23 induces heart hypertrophy in sickle cell disease. Haematologica 102: e33-e35, 2017.
73. Miikkulainen P, Högel H, Rantanen K, Suomi T, Kouvonen P, Elo LL and Jaakkola PM: HIF prolyl hydroxylase PHD3 regulates translational machinery and glucose metabolism in clear cell renal cell carcinoma. Cancer Metab 5: 5, 2017.
74. Ivan M and Kaelin WG Jr: The EGLN-HIF O_2-sensing system: Multiple inputs and feedbacks. Mol Cell 66: 772-779, 2017.
75. Pugh CW and Ratcliffe PJ: New horizons in hypoxia signaling pathways. Exp Cell Res 356: 116-121, 2017.
76. Prabhakar NR and Semenza GL: Oxygen sensing and homeostasis. Physiology (Bethesda) 30: 340-348, 2015.
77. Maxwell PH and Eckardt KU: HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. Nat Rev Nephrol 12: 157-168, 2016.
78. Wyatt CM and Drüeke TB: HIF stabilization by prolyl hydroxylase inhibitors for the treatment of anemia in chronic kidney disease. Kidney Int 90: 923-925, 2016.
79. Kanbay M, Vervloet M, Cozzolino M, Siriopol D, Covic A, Goldsmith D and Solak Y: Novel faces of fibroblast growth factor 23 (FGF23): Iron deficiency, inflammation, insulin resistance, left ventricular hypertrophy, proteinuria and acute kidney injury. Calcif Tissue Int 100: 217-228, 2017.
80. Agoro R, Ni P, Noonan ML and White KE: Osteocytic FGF23 and its kidney function. Front Endocrinol (Lausanne) 11: 592, 2020.
81. Provenzano R, Besarab A, Sun CH, Diamond SA, Durham JH, Cangiano JL, Aiello JR, Novak JE, Lee T, Leong R, *et al*: Oral hypoxia-inducible factor Prolyl hydroxylase inhibitor roxadustat (FG-4592) for the treatment of anemia in patients with CKD. Clin J Am Soc Nephrol 11: 982-991, 2016.
82. Hanudel MR, Laster M and Salusky IB: Non-renal-Related Mechanisms of FGF23 Pathophysiology. Curr Osteoporos Rep 16: 724-729, 2018.
83. Wheeler JA and Clinkenbeard EL: Regulation of fibroblast growth factor 23 by iron, EPO, and HIF. Curr Mol Biol Rep 5: 8-17, 2019.

84. Zhang Q, Doucet M, Tomlinson RE, Han X, Quarles LD, Collins MT and Clemens TL: The hypoxia-inducible factor-1α activates ectopic production of fibroblast growth factor 23 in tumor-induced osteomalacia. Bone Res 4: 16011, 2016.
85. Babitt JL and Sitara D: Crosstalk between fibroblast growth factor 23, iron, erythropoietin, and inflammation in kidney disease. Curr Opin Nephrol Hypertens 28: 304-310, 2019.
86. Khozeymeh F, Mortazavi M, Khalighinejad N, Akhavankhaleghi M and Alikhani M: Salivary levels of interleukin-6 and tumor necrosis factor-α in patients undergoing hemodialysis. Dent Res J (Isfahan) 13: 69-73, 2016.
87. Wu J, Guo N, Chen X and Xing C: Coexistence of micro-inflammatory and macrophage phenotype abnormalities in chronic kidney disease. Int J Clin Exp Pathol 13: 317-323, 2020.
88. Kim IY, Kim JH, Kim MJ, Lee DW, Hwang CG, Han M, Rhee H, Song SH, Seong EY and Lee SB: Low 1,25-dihydroxyvitamin D level is associated with erythropoietin deficiency and endogenous erythropoietin resistance in patients with chronic kidney disease. Int Urol Nephrol 50: 2255-2260, 2018.
89. Icardi A, Paoletti E, De Nicola L, Mazzaferro S, Russo R and Cozzolino M: Renal anaemia and EPO hyporesponsiveness associated with vitamin D deficiency: The potential role of inflammation. Nephrol Dial Transplant 28: 1672-1679, 2013.
90. Lee B, Kwon E, Kim Y, Kim JH, Son SW, Lee JK, Kim DW, Sohn J, Kim TH and Ji JD: 1α,25-Dihydroxyvitamin D3 upregulates HIF-1 and TREM-1 via mTOR signaling. Immunol Lett 163: 14-21, 2015.
91. van Vuren AJ, Gaillard C, Eisenga MF, van Wijk R and van Beers EJ: The EPO-FGF23 signaling pathway in erythroid progenitor cells: Opening a new area of research. Front Physiol 10, 304, 2019.
92. Yousaf F and Spinowitz B: Hypoxia-inducible factor stabilizers: A new avenue for reducing BP while helping hemoglobin? Curr Hypertens Rep 18: 23, 2016.
93. Honda H, Michihata T, Shishido K, Takahashi K, Takahashi G, Hosaka N, Ikeda M, Sanada D and Shibata T: High fibroblast growth factor 23 levels are associated with decreased ferritin levels and increased intravenous iron doses in hemodialysis patients. PLoS One 12: e0176984, 2017.
94. Artunc F and Risler T: Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease. Nephrol Dial Transplant 22: 2900-2908, 2007.
95. Smith ER, Cai MM, McMahon LP and Holt SG: Biological variability of plasma intact and C-terminal FGF23 measurements. J Clin Endocrinol Metab 97: 3357-3365, 2012.
96. Shimada T, Urakawa I, Isakova T, Yamazaki Y, Epstein M, Wesseling-Perry K, Wolf M, Salusky IB and Jüppner H: Circulating fibroblast growth factor 23 in patients with end-stage renal disease treated by peritoneal dialysis is intact and biologically active. J Clin Endocrinol Metab 95: 578-585, 2010.
97. Kalyanasundaram A and Fedorov VV: Fibroblast growth factor 23: A novel key to find hidden substrates of atrial fibrillation? Circulation 130: 295-297, 2014.