FGF23, IRON STATUS AND VITAMIN D METABOLISM IN CHRONIC KIDNEY DISEASE

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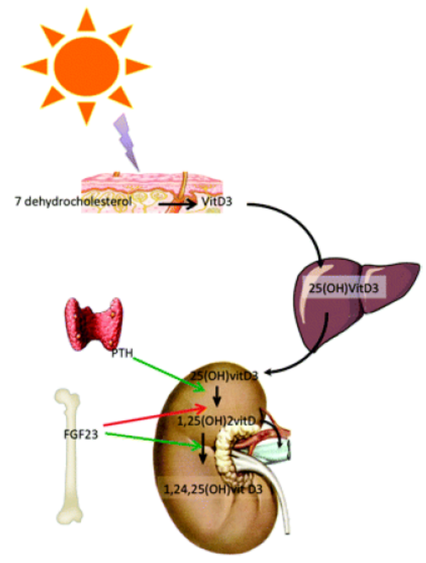
Introduction

Fibroblast growth factor-23 (FGF23) is a major regulator of phosphate and vitamin D metabolism often elevated in genetic hypophosphataemic disorders and in chronic kidney disease. In the kidney, FGF23 induces urinary phosphate excretion and reduces synthesis of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by down regulating 1α-hydroxylase and upregulating 24-hydroxylase activity. We[1] and others[2,3] showed a relationship between FGF23 and various markers of iron status including ferritin, low serum iron being associated with elevated c-terminal (cFGF23) but not intact (iFGF23) FGF23 suggesting a role of iron status in the metabolism of FGF23. Moreover in dialysed patients with chronic kidney disease (CDK), iron administration lowers iFGF23 levels[4].



from Prié and Friedlander CJASN 2010[5]

Objectives:

- Compare the new Biomedica with the Immunotopics assay for cFGF23 (routinely used in our laboratory).
- Determine ferritin status in CKD patients.
- Determine vitamin D metabolism in CKD patients.

Methods

❖ Samples

Comparison: Samples with a range of concentrations of cFGF23 (11-3600 RU/mL).

CKD: Randomized samples from patients with chronic kidney disease (CKD; eGFR < 70 ml/min/1.73 m2) and controls (eGFR >100 ml/min/1.73 m2).

Samples were anonymised to the researchers at point of access in accordance with generic ethical approval

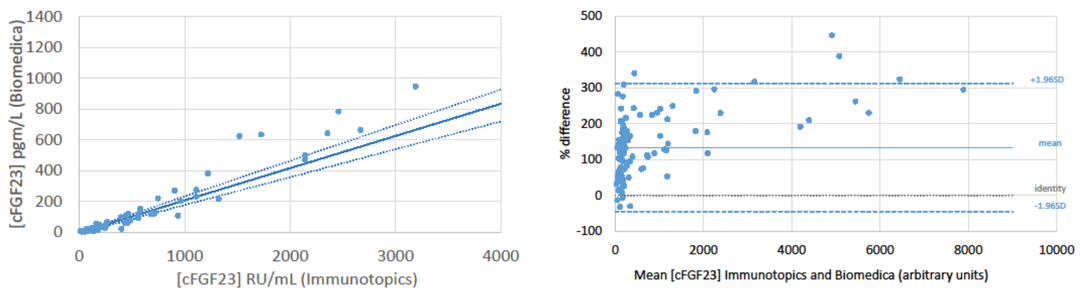
❖ Assays

- c-terminal FGF23 (cat# 60-6100) and intact FGF23 (cat# 60-6600) were two-site enzyme-linked immunosorbent assay (ELISA) 2nd generation from Immunotopics Inc., CA.
- c-terminal FGF23 (cat# BI-20702) was a sandwich enzyme immunoassay from Biomedica. Concentrations were calculated using 1pmol/L = 0.133pg/mL.
- ferritin (cat# 03737551), urea (cat# 04460715) and creatinine (cat# 04810716) were immunoassay or kinetic colorimetric assays by Roche Diagnostics (Burgess Hill, UK) measured on a COBAS 6000.
- 25 hydroxyvitamin D ($25(OH)D_2$ and $25(OH)D_3$) and it's metabolite 24,25 dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) were measured by LC-MS/MS.

❖ Statistics

- Assay results were compared using Passing Bablock and Bland-Altman analyses.
- Concentrations were compared using one-way ANOVA. Trends were estimated using linear regression analysis
- SPSS for windows version 22.0.0.1 was used and results were considered statistically significant for $p<0.05$ [*$p<0.05$; ** $p<0.01$; ***$p<0.001$].

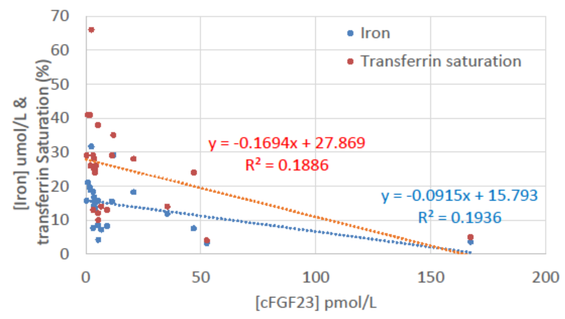
FGF23 assay comparisons



Passing-Bablock between the different cFGF23 assays
Line represents the best fit; dotted lines represent 95% confidence interval

The assay currently in routine use in our laboratory to assess FGF23 status is the cFGF23 assay from Immunotopics. We compared results from a newly available kit from Biomedica which showed good correlation (n = 125 *r* = 0.966 $p<0.01$) however, a bias became apparent in the highest range of FGF23.

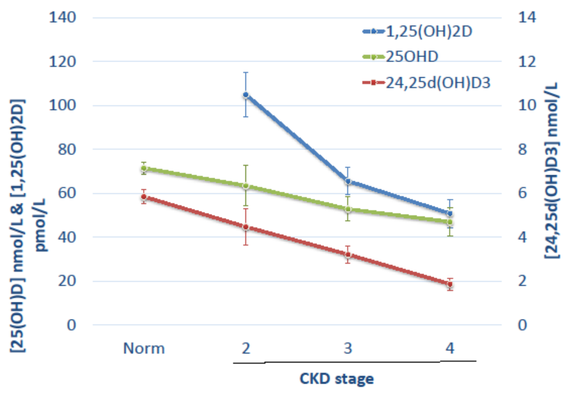
FGF23 and Iron metabolism in CKD



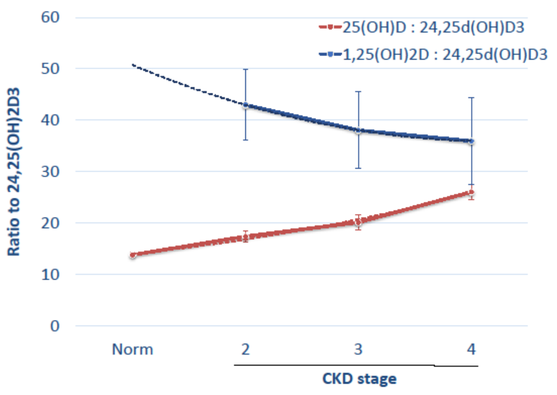
Significant negative correlations were observed between cFGF23 and both iron concentration (Pearson's r=-0.44 $p<0.05$) and transferrin saturation (r=-0.434 $p<0.05$).

Correlation between cFGF23 concentrations and iron metabolism in CKD patients

Vitamin D and FGF23 in CKD



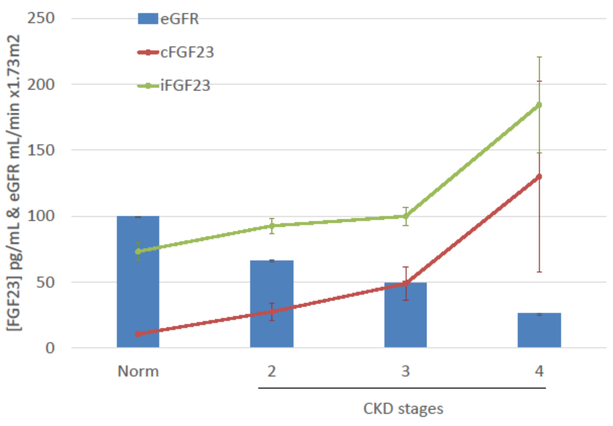
Comparison of the grand means (±SEM) of serum 25(OH)D, serum $1,25(OH)_2D$ and $24,25(OH)_2D3$ between CKD patients (n=74, stage 2 to 4) and non-CKD (n=79) controls.



Comparison of the ratios of serum 25(OH)D and serum $1,25(OH)_2D$ to $24,25(OH)_2D3$ between CKD patients (n=74, stage 2 to 4) and non-CKD (n=79) controls.

Decreased concentrations of 25(OH)D, 1,25(OH)2D and $24,25(OH)_2D$. Increase ratio [25(OH)D]:[$24,25(OH)_2D3$]. Concentrations of FGF23, both C-terminal and intact, increased with decreasing kidney function. Both iFGF23 and cFGF23 correlated with the ratio 25(OH)D:24,25(OH)2D3 (Pearson's rho = 0.190 and 0.204, $p<0.05$, respectively) and iFGF23 also significantly correlated with 24,25(OH)2D3 (Pearson's rho = -0.323 $p<0.01$)

FGF23 in CKD



Parallel increase of intact and c-terminal FGF23 concentrations as eGFR decreased especially in patients with end-stage renal disease (stage 4 and over) usually regarded as a compensatory response to hyperphosphatemia or phosphate overload.

Graphs showing the concentrations of c-terminal and intact FGF23 in CKD stage 2 to 4.

Conclusions

❖ The cFGF23 assay from Biomedica correlates well with the assay from Immutopics.

❖ The assays measure both cFGF23 and iFGF23, however we observed lower concentrations of cFGF23 than iFGF23, suggesting that the assays are measuring different forms of the protein and/or the specificity of the antibody used is different.

❖ We observed in patients with relatively low ferritin higher levels of cFGF23 which is in accordance with previous studies in FGF23 related diseases. Iron deficiency has been shown to be associated with stimulation of FGF23 transcription and increased concentrations of cFGF23 and iFGF23 in patients with dominant hypophosphatemic rickets while iFGF23 are maintained in controls[2].

Iron and FGF23 may act in concert to regulate vitamin D status, more studies are needed to make conclusions on the diagnostic value of these results. Iron and/vitamin D supplementation might decrease or increase the observed effects.

❖ We observed vitamin D deficiency in CKD patients associated with an overall decrease in $24,25(OH)_2D3$ concentration as shown previously[6].

❖ The ratio of 25(OH)D: 24,25(OH)2D is markedly elevated and increases as CKD progresses suggesting a relatively lower catabolic rate of 25(OH)D towards its $24,25(OH)_2D$ metabolite . This may be in an attempt to allow ongoing synthesis of 1,25(OH)2D continuing its biological effects. The significant correlations of FGF23 with the $24,25(OH)_2D3$ and the ratio 25(OH)D:$24,25(OH)_2D3$ suggest a potential role for FGF23 in the regulation of 24-hydroxylase in CKD. It would appear that the effects of FGF23 on vitamin D metabolism in CKD are greater than the effects of PTH and so this data adds further to the proposals that the early management and prevention of the increase of FGF23 in CKD may be beneficial in preventing CKD-BMD.

References:

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