Evaluation of the relationship between clinical parameters of mineral and bone disease, fibroblast growth factor 23, and related medication use among hemodialysis patients

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Evaluation of the Relationship between Clinical Parameters of Mineral and Bone Disease, Fibroblast Growth Factor 23, and Related Medication Use Among Hemodialysis Patients

An abstract of a thesis presented to the Faculty of the University of Albany, State University of New York in partial fulfillment of the requirements for the degree of

Master of Science
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ABSTRACT

Background

Fibroblast growth factor 23 (FGF-23) is a key regulator of mineral metabolism and has been associated with adverse clinical outcomes in patients with kidney disease. Limited data exists regarding the relationship between medications used to treat mineral and bone disorder (MBD) and FGF-23 in the dialysis population.

Methods

This was a secondary analysis of data derived from a previously conducted cross-sectional study (Vitamin D Receptor Gene on Vitamin D Dosing and Clinical Response in Stage 5D Chronic Kidney Disease [VDR PGX] Study) by our group. This dataset contains de-identified demographic, clinical, genetic and biomarker data for over 100 hemodialysis patients recruited from Rubin Dialysis Centers (Clifton Park, Troy and Saratoga, NY). NY). The VDR PGX Study enrolled adult hemodialysis patients (≥ 18 years of age) who were on chronic dialysis treatment for at least 3 months. Correlation and stepwise linear regression were used to identify factors (i.e. demographic, clinical lab parameters, medications) associated with FGF-23.

Results

Log FGF-23 levels were higher among patients taking cinacalcet, n = 38, compared to those not taking cinacalcet, n = 80, (2.62 ± 0.41 pg/ml versus 2.44 ± 0.31 pg/ml, P = 0.0185). Use of cinacalcet (β = 0.14, P = 0.0052), calcium (β = 0.19, P < 0.0001), phosphorus (β = 0.11, P < 0.0001) and dialysis vintage (β = 0.27, P = 0.0013) were independently associated with log FGF-23. However, patients taking cinacalcet had higher PTH levels and were receiving greater doses of doxercalciferol.
Conclusions

The observed relationship between cinacalcet use and FGF-23 appeared to be a function of secondary hyperparathyroidism disease severity, as indicated by the presence of higher PTH levels and the use of larger doses of activated vitamin D among patients on cinacalcet therapy. The impact of cinacalcet on FGF-23 in severe secondary hyperparathyroidism warrants further investigation.
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CHAPTER 1: INTRODUCTORY CHAPTER
Chronic Kidney Disease

Chronic kidney disease (CKD) is a progressive disorder characterized by irreversible kidney damage resulting in diminished renal function. The best measure of kidney function is glomerular filtration rate (GFR), the rate at which fluid is filtered through the kidney. In the clinical setting, renal function is determined using the Modification of Diet in Renal Disease (MDRD) equation to estimate GFR. A diagnosis of CKD is made based upon the presence of kidney damage or reduced kidney function. Specifically, CKD is defined as kidney damage (structural or functional abnormalities of the kidney present for 3 months or greater) or a GFR of less than 60 mL/min/1.73 m² for 3 months or greater with or without kidney damage. CKD is further classified into stages, ranging from stage 1 to stage 5 disease (Table 1). Stage 5 CKD, also termed end-stage renal disease (ESRD), is the most severe stage of CKD. Patients who progress to CKD stage 5 require chronic renal replacement therapy in the form of dialysis or kidney transplantation to sustain life. As CKD progresses, secondary complications become more prevalent (i.e. anemia, electrolyte abnormalities, disordered mineral metabolism, malnutrition).

Table 1. Stages of Chronic Kidney Disease

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or ↑ GFR</td>
<td>≥ 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild ↓ GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate ↓ GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severe ↓ GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt; 15 or dialysis</td>
</tr>
</tbody>
</table>
**Definition of Chronic Kidney Disease Mineral and Bone Disorder**

Chronic Kidney Disease Mineral and Bone Disorder (CKD-MBD) is a systemic disorder of mineral and bone metabolism due to CKD and is manifested by any one or a combination of the following: abnormalities of calcium, phosphorus, parathyroid hormone (PTH) or vitamin D metabolism; abnormalities in bone turnover, mineralization, volume, linear growth, or strength; the presence of vascular or other soft tissue calcification. As renal function declines, derangements in calcium, phosphorus, PTH and vitamin D metabolites become increasingly prevalent. Abnormalities of mineral metabolism (i.e. hyperphosphatemia, hypocalcemia, hyperparathyroidism, decreased production of activated vitamin D) begin to appear as early as stage 3 disease and are nearly universal in the dialysis population. These derangements lead to secondary complications of CKD-MBD (i.e. osteodystrophy, vascular and soft tissue calcification) putting those with kidney disease at a higher risk of death, compared to the general population.

**PTH**

PTH is synthesized and released from the parathyroid glands. The primary physiologic role of this hormone is maintenance of calcium homeostasis by three mechanisms: 1) increasing bone mineral dissolution, thus releasing calcium and phosphorus; 2) increasing the renal absorption of calcium and renal elimination of phosphorus; and 3) enhancing intestinal absorption of calcium and phosphorus indirectly through its effects on activated vitamin D (1,25-dihydroxyvitamin D) synthesis. In a hypocalcemic state, low levels of serum calcium are detected by calcium sensing receptors located on the parathyroid gland. PTH is subsequently secreted, restoring serum
calcium levels while maintaining normal phosphorus concentrations. Extracellular phosphate also has direct effects on PTH.

**Vitamin D**

The term “vitamin D” is a generic descriptor referring to both the D$_2$ ( ergocalciferol) and D$_3$ (cholecalciferol) forms. Vitamin D is obtained from dietary sources and is also synthesized by body. The main exogenous sources in the western diet are: 1) vitamin D$_2$ from plants; 2) vitamin D$_3$ from oily fish; and 3) vitamin D supplemented milk.$^4$ However, only vitamin D$_3$ is synthesized by the human body. Within the stratum spnosum and stratum basale layers of the skin, 7-dehydrocholesterol reacts with ultraviolet sunlight producing vitamin D$_3$.

Vitamin D requires two hydroxylations to become bioactive. Once in the bloodstream, vitamin D binds to vitamin D binding protein and is carried to the liver where it is converted to 25-hydroxyvitmain D by the enzyme 25-hydroxylase. Subsequently, 25-hydroxyvitamin D is then converted to 1,25-dihydroxyvitamin D (the biological active form of vitamin D) by the action of the enzyme 1-α hydroxylase in the kidneys. It should be noted that PTH enhances 1,25-dihydroxyvitamin D production by means of renal 1-α hydroxylase stimulation.

1,25-dihydroxyvitamin D, circulates in the bloodstream bound to vitamin D binding protein and enters target cells where it interacts with its nuclear vitamin D receptor. The hormonal functions of 1,25-dihydroxyvitamin D are carried out in two main target organs: 1) the intestines, where it regulates the absorption of calcium and phosphorus; and 2) the parathyroid glands, where it inhibits PTH synthesis at the level of messenger RNA transcription.$^4$
FGF-23

The recently discovered bone-derived hormone FGF-23 is a key regulator of phosphate homeostasis and activated vitamin D production. Osteoblasts and osteocytes secrete FGF-23 in response to elevated serum concentrations of phosphorus and 1,25-dihydroxyvitamin D. To restore phosphorous homeostasis, FGF-23 promotes urinary phosphorus excretion through down-regulation of sodium-phosphate co-transporters located in renal proximal tubules. Additionally, this hormone regulates activated vitamin D production via inhibition of renal 1-α hydroxylase and up-regulation the enzyme responsible for catabolism of activated vitamin D, of 24-hydroxylase. FGF-23 also mediates PTH secretion indirectly, through its effects on phosphorus excretion and vitamin D metabolism and directly acts on the parathyroid gland to decrease PTH synthesis and secretion.5,6

Pathophysiology of CKD-MBD

Abnormalities in Mineral Metabolism

CKD-MBD is a complex co-morbid state characterized by disturbances in phosphorus, calcium, vitamin D and PTH concentrations. The “trade-off” hypothesis best describes the pathogenesis of CKD-MBD. As renal function declines the ability of the kidneys to excrete phosphorus and produce activated vitamin D is diminished. The ensuing abnormalities (hyperphosphatemia and reduced circulating concentrations of activated vitamin D, respectively) have two main effects, subsequent elevations in FGF-23 and PTH levels.

Beginning in early CKD (stages 1 and 2) FGF-23 seems to function as a protective factor, it is secreted to maintain normal phosphorus balance by increasing
urinary phosphosphate excretion. However, elevations in FGF-23 necessary to maintain phosphorus homeostasis come at a cost, activated vitamin D deficiency via the inhibitory actions of FGF-23 on 1-α hydroxylase; the “trade-off.” Typically in the early stages of CKD, the PTH stimulatory effects of FGF-23 induced vitamin D deficiency is counterbalanced by the inhibitory actions of FGF-23 on the parathyroid gland; resulting in normal PTH levels or only modest elevations.

As kidney disease progresses to the latter stages (CKD stages 3, 4 and 5), hyperphosphatemia develops. The inability of failing or failed kidneys to eliminate phosphorus, despite tremendous increases in FGF-23, in combination with a constant phosphate intake from the diet cause an overt increase in serum phosphorus levels. In hyperphosphatemic conditions, excess phosphorus chelates ionized calcium leading to hypocalcemia; PTH is constantly secreted (secondary hyperparathyroidism [SHPT]) as effort to maintain calcium homeostasis, a “trade off.” In addition to hyperphosphatemia, extreme elevations in FGF-23, secreted in an effort to eliminate excess phosphorus, indirectly contributes to the pathogenesis of SHPT. FGF-23 induced 1,25-dihydroxyvitamin D deficiency promotes hypocalcemia via reduced intestinal calcium absorption. Furthermore, decreased suppression of PTH release due to activated vitamin D deficiency contributes to the pathogenesis of SHPT.

**Bone Abnormalities**

Changes in bone structure develop early in the course of CKD and worsen as kidney disease progresses. The magnitude of these changes can be influenced by various therapeutic interventions, such as vitamin D administration. Renal osteodystrophy refers
to the bone disorders that develop as a result of disordered mineral metabolism in CKD; osteitis fibrosa cystica, osteomalacia, and adynamic bone disease.\(^4\)

Osteitis fibrosa cystica, “high-turnover bone disease”, is caused by elevated PTH concentrations which stimulate osteoclast activity, bone breakdown, and resorption. In osteitis fibrosa cystica, bones become soft putting patients at a higher risk for fracture. The most common symptom of this disorder is generalized bone pain. Osteomalacia, “soft bone”, is caused by a lack of vitamin D and is characterized by low bone turnover and abnormal mineralization. Similar to osteitis fibrosa cystica, patients with osteomalacia may present with generalized bone pain and are at a higher risk of fracture. Adynamic bone disease, “low-turnover bone disease”, is a medically induced syndrome caused by over-suppression of the parathyroid gland, inhibiting PTH release. Adynamic bone disease is characterized by a lack of bone cell activity and markedly reduced bone turnover. Patients with adynamic bone disease are often asymptomatic; however like the other forms of bone disease patients with adynamic bone disease are more susceptible to fracture.

**Soft Tissue and Vascular Calcification**

Vascular calcification causes increased arterial stiffness, left ventricular hypertrophy, decreased coronary artery perfusion, myocardial ischemia, and increased cardiovascular morbidity and mortality.\(^7\) Disordered mineral metabolism, common in CKD, predisposes patients to the development of vascular and soft tissue calcification. Historically, vascular and soft tissue calcification in CKD was considered to occur predominantly by a passive physicochemical mechanism; super-saturation of phosphorus in the plasma leading to passive calcium-phosphate deposition in extra-skeletal tissues.\(^8\)
Now, however, calcification is considered to be a regulated process influenced by tissue-specific cellular mechanisms; loss of inhibitors (e.g. fetuin-A, matrix Gla-protein, osteopontin, pyrophosphate, osteoprotegerin) that usually prevent tissue calcification due to the chronic inflammatory milieu of uremia. Additionally, hyperphosphatemic conditions induce transformation of vascular smooth muscle cells into bone-like initiating process of blood vessel calcification; these bone-like cells produce a matrix of bone collagen and noncollagenous proteins involved in bone metabolism.

**FGF-23 and Clinical Outcomes**

Initially it was unclear whether higher FGF-23 levels were protective or harmful in CKD due to the hormone’s ability to prevent phosphorus accumulation and exacerbate 1,25-dihydroxyvitamin D deficiency, respectively. However, current data provide evidence that even though excess FGF-23 may be important for maintenance of phosphate homeostasis in kidney disease, long-term exposure to elevated FGF-23 may in fact be maladaptive and lead to poor patient outcomes. Numerous observational studies have shown that, higher circulating concentrations of FGF-23 have been associated with the presence of more severe vascular calcification, left ventricular hypertrophy, atherosclerotic burden, endothelial dysfunction, cardiovascular events and mortality. Currently, the optimal level of FGF-23 which will maximize patient outcomes is unknown. Furthermore, the causal nature of these associations and possible biologic mechanisms still needs to be elucidated.

**Impact of CKD-MBD Therapies on FGF-23**

Medications used in the treatment of CKD-MBD affect FGF-23. Specifically, phosphate binders, activated vitamin D and cinacalcet influence circulating FGF-23
levels. It is important to understand how current medication therapy and clinical practices affect FGF-23. Given the significant role of FGF-23 in the regulation of mineral metabolism, understanding how these therapies modulate FGF-23 while successfully controlling clinical parameters of CKD-MBD may lead to better clinical outcomes in the dialysis population.

Activated Vitamin D

Activated vitamin D (i.e. calcitriol) and activated vitamin D analogs (i.e. paricalcitol and doxercalciferol) are the primary agents used to control SHPT. These agents bind to the vitamin D receptor on the parathyroid gland. Intracellular signaling results in decreased production of PTH messenger RNA which leads to reduced PTH secretion, and thereby reduces serum PTH levels.

1,25-dihydroxyvitamin D directly stimulates FGF-23 expression in osteocytes. In ESRD, several clinical studies have illustrated that activated vitamin D administration is associated with subsequent elevations in FGF-23 levels, possibly in a dose dependent manner. Numerous observational studies have demonstrated that dialysis patients treated with calcitriol or vitamin D analogs have a lower risk of death. However, beneficial effects of these agents on survival is reduced and may be related to the FGF-23 stimulatory effects of these drugs.

Currently, there is only one published trial comparing effects of different vitamin D analogs on FGF-23 in humans. Pediatric peritoneal dialysis patients were randomized to treatment with oral calcitriol or doxercalciferol and a phosphate binder for a total of 8 months. Therapy with calcitriol and doxercalciferol resulted in similar increases in FGF-23, irrespective of phosphate binder selection.
Nutritional Vitamin D

It remains unknown if vitamin D supplementation with nutritional vitamin D compounds (i.e. the 25-hydroxyvitamin D compounds ergocalciferol and cholecalciferol) stimulates FGF-23 secretion like 1,25-dihydroxyvitmain D compounds.

Cinacalcet

Calcimimetic therapy is frequently initiated, on top of vitamin D therapy in patients with persistent SHPT (i.e. those with poor control of PTH despite the use vitamin D analogs). Currently, the only calcimimetic on the market is cinacalcet. Cinacalcet is an allosteric modulator of calcium sensing receptors located on parathyroid glands which increases the sensitivity of these receptors to extracellular calcium. As a result, PTH secretion is inhibited and serum PTH levels will decrease.

Data from both animal and human studies suggest that cinacalcet therapy may in fact reduce circulating FGF-23. However, it remains unclear if cinacalcet directly affects FGF-23 or indirectly regulates FGF-23 via changes in other clinical parameters of mineral and bone disorder. Uremic rats treated for 6 weeks with 15 mg/kg of cinacalcet had lower serum levels of FGF-23 levels compared to controls. Of note, the reduction in circulating FGF-23 concentrations observed in cinacalcet treated rats was accompanied by a simultaneous decrease in 1,25-dihydroxyvitmain D, a known stimulus of FGF-23 production. In addition, a recent open-label, single arm clinical trial conducted in hemodialysis patients with SHPT demonstrated that cinacalcet lowered FGF-23 levels, independent of vitamin D analogs. However, concomitant reductions in serum calcium, phosphorus and PTH were also observed.
A secondary analysis of the ACHIEVE trial showed that treatment with cinacalcet plus fixed low-dose vitamin D analogs (VDAs), the Cinacalcet-D group, compared to the use of titrated VDAs alone (Flex-D) resulted in significantly lower FGF-23 levels after 27 weeks of treatment. Subjects in the Flex-D group received roughly 2.5 times the average weekly dose of VDA therapy compared to Cinacalcet-D patients. Essentially, the use of titratable cinacalcet in combination with fixed low-dose VDAs allowed for a VDA sparing effect while providing similar PTH control, accounting for the lower FGF-23 levels in Cinacalcet-D group.

**Phosphate Binders**

Phosphate binding agents are initiated in ESRD to control the high phosphorus levels often present. In the earlier stages of kidney disease, phosphorus levels can typically be controlled via dietary phosphorus restriction. However, in the latter stages of the disease (stages 4 and 5) a significant reduction of serum phosphorus cannot be achieved with dietary interventions alone. Thus, medical intervention in the form of phosphate binding medications is often required in addition to dietary changes. Currently, there are two main classes of phosphate binding medications on the market, calcium based (calcium acetate and calcium carbonate) and non-calcium based binders (sevelamer hydrochloride, sevelamer carbonate and lanthanum carbonate).

In patients with CKD, higher phosphorus exposure has been associated with increases in FGF-23 concentrations. Whereas reducing phosphate load, either by dietary phosphorus restriction or use phosphate binding agents, lowers FGF-23 levels. However, significant reductions in FGF-23, after treatment with phosphate binders, are
not apparent after short term treatment (i.e. two weeks or less) indicating that continuous therapy long term is required for FGF-23 suppression.

Recent data indicate that the use of sevelamer (a non-calcium based phosphate binder) results in greater reductions of FGF-23 compared to calcium based phosphate binders. A post-hoc analysis of the BRIC study demonstrated that use of sevelamer for 1 year resulted in a significant decrease in FGF-23 from baseline in hemodialysis patients, whereas a non-significant decrease was observed in patients treated with calcium acetate. These results are similar to a study in pre-dialysis CKD patients which found that short-term treatment with sevelamer resulted in a significant decrease in FGF-23 compared to patients receiving calcium acetate. At this point in time, there is limited data demonstrating the FGF-23 lowering abilities of lanthanum carbonate (a non-calcium based phosphate binder) and there is only one ongoing clinical trial comparing the effects of lanthanum carbonate versus calcium acetate on circulating FGF-23 levels (NCT01357317).
CHAPTER 2: SCIENTIFIC ARTICLE

Introduction

CKD-MBD is characterized by disturbances in phosphorus, calcium, vitamin D and PTH. The recently discovered bone-derived protein FGF-23, an important regulator of mineral metabolism, plays a key role in the development and potentiation of CKD-MBD. Numerous clinical investigations have demonstrated strong correlations between serum phosphate, calcium, 1,25-dihydroxyvitamin D and PTH and FGF-23 levels in dialysis patients. In addition, higher circulating concentrations of this hormone have been associated with the presence of more severe vascular calcification, left ventricular hypertrophy and mortality in the hemodialysis population.

FGF-23 promotes phosphate excretion via down-regulation of sodium-phosphate co-transporters in the renal tubules, and decreases 1,25-dihydroxyvitamin D concentrations by means of renal 1-α hydroxylase inhibition and 24-hydroxylase stimulation. Additionally, FGF-23 mediates parathyroid hormone secretion indirectly, via its effects on phosphorus excretion and vitamin D metabolism, and directly acts on the parathyroid gland to decrease PTH synthesis and secretion. As renal function declines, circulating levels of FGF-23 increase in response to worsening hyperphosphatemia. This elevation in FGF-23 leads to decreased production of activated vitamin D, and as a result FGF-23 can contribute to the development of secondary hyperparathyroidism (SHPT). In end stage renal disease, overt hyperphosphatemia, due to phosphate retention, and activated vitamin D administration lead to remarkably high circulating FGF-23 concentrations. Often, in dialysis patients, PTH levels remain elevated despite exceptionally high FGF-23 concentrations indicating resistance of the parathyroid gland to FGF-23 in this setting.
Therapies used in the treatment of CKD-MBD such as phosphate binders, activated vitamin D and cinacalcet can influence circulating FGF-23 levels. Specifically, withdrawal of phosphate binders, calcitriol administration, use of calcium acetate compared to sevelamer, as well as use of dialysate with higher calcium concentrations have been associated with higher circulating concentrations of FGF-23, respectively. Whereas cinacalcet administration or co-administration of fixed low-dose activated vitamin D analogs (VDAs) plus cinacalcet (compared to the use of titrated VDAs alone) were associated with lower FGF-23 levels.

Given the significant role of FGF-23 in mineral metabolism and in the pathogenesis of SHPT, understanding how therapies modulate FGF-23 while successfully controlling clinical parameters of CKD-MBD may lead to better clinical outcomes in the dialysis population. Furthermore, it is important to understand how current medication therapy and clinical practices affect FGF-23. The aim of this investigation was to explore the relationship between FGF-23, clinical parameters of CKD-MBD and related medication use in hemodialysis patients.

Materials and Methods

Study design and subjects

This was a secondary analysis of data derived from a previously conducted cross-sectional study (Vitamin D Receptor Gene on Vitamin D Dosing and Clinical Response in Stage 5D Chronic Kidney Disease [VDR PGX] Study) by our group. This dataset contains de-identified demographic, clinical, genetic and biomarker data for over 100 hemodialysis patients recruited from Rubin Dialysis Centers (Clifton Park, Troy and Saratoga, NY). Briefly, the patients were eligible to enroll in the VDR PGX study if they
were 18 years of age or older and had been on chronic hemodialysis for at least 3 months. This secondary analysis received an Institutional Review Board (IRB) exemption from the Albany College of Pharmacy and Health Sciences IRB.

**Statistical analysis**

Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). A P value < 0.05 was considered significant for all two-tailed tests, unless specified otherwise. Data were reported as mean ± standard deviation. Serum calcium levels were adjusted for albumin and are reported as corrected calcium. All weekly intravenous VDA doses were converted to doxercalciferol equivalent doses for uniformity (3.1:4.6 for doxercalciferol:paricalcitol and 3.1:1 for doxercalciferol:calcitriol) due to the fact that the vast majority (96%) of patients on intravenous VDA therapy were using doxercalciferol. A log transformation was used to normalize the skewed distributions of FGF-23 and intact PTH.

The linear relationship between log FGF-23 and each normally distributed continuous parameter were evaluated using Pearson’s correlation. Comparisons of FGF-23 concentrations between levels of categorical variables were made using Student’s t-test, Wilcoxon rank-sum test, ANOVA or the Kruskal-Wallis test where appropriate. Stepwise linear regression modeling, using a backward elimination strategy, was used to determine factors independently associated with log FGF-23 concentrations. All variables with a P value ≤ 0.1 on bivariate analyses were included in the preliminary multivariate linear regression model. Interaction between all potential covariates and specific medication use/nonuse were assessed by including interactive term with age, dialysis vintage, corrected calcium, phosphorus and log intact PTH. All interactive terms and
independent variables which maintained a P-value < 0.05 were retained in the final multivariate linear regression model and considered significant predictors. In addition, all interactive terms and independent variables were assessed as potential confounders. A covariate which induced a greater than 10% change in the β estimates, upon removal, was considered to be a confounder and was retained in the model. Comparisons of continuous variables between subjects on cinacalcet therapy to those not receiving cinacalcet were made using Student’s t-test. Pearson’s χ² test and Fisher’s exact test were used to analyze categorical variables where appropriate.

Results

A total of 118 hemodialysis patients were studied. The characteristics of the study population are shown in Table 2. Demographic and clinical laboratory parameters were evaluated to examine their relationship with log FGF-23. Log FGF-23 negatively correlated with age (r = -0.27, P = 0.0026) and positively correlated with dialysis vintage (r = 0.26, P = 0.0041), corrected calcium (r = 0.25, P = 0.0063), phosphorus (r = 0.56, P < 0.0001) and log intact PTH (r = 0.31, P = 0.0006). Log FGF-23 did not correlate with albumin, hemoglobin, ferritin or transferrin saturation. There was no difference in log FGF-23 levels between the sexes (P = 0.7493) or races (Caucasian versus other, P = 0.3456).

The relationship between log FGF-23 and common medications used in the treatment of CKD-MBD was evaluated. Subjects taking cinacalcet (n = 38) had higher log FGF-23 levels compared to those not taking cinacalcet (n = 80), 2.63 ± 0.41 pg/mL versus 2.44 ± 0.31 pg/mL, P = 0.0185. Among patients taking phosphate binders, phosphate control and log FGF-23 levels were similar between those using calcium based
binders and non-calcium based binders. Log FGF-23 concentrations did not differ between patients receiving intravenous VDA therapy (n = 83) compared to subjects not receiving VDAs, or between patients receiving ergocalciferol (n = 42) compared to those not receiving ergocalciferol.

Stepwise linear regression modeling was used to identify factors associated with log FGF-23 levels (Table 3). Of the independent variables included in the preliminary model, cinacalcet use (P = 0.0052), dialysis vintage (P = 0.0013), corrected calcium (P < 0.0001) and phosphorus (P < 0.0001) were significantly associated with log FGF-23 concentrations and were retained in the final model. There was no additive interaction present between these variables and ergocalciferol use, intravenous vitamin D use or phosphate binder use. These independent variables accounted for 50.4 % of the variance of log FGF-23 concentrations.

The association between log FGF-23 and cinacalcet use was further analyzed (Table 4). Subjects on cinacalcet therapy were taking an average of 51.5 ± 41.3 mg per day for 6.9 ± 6.3 months. They were younger (P = 0.0085), had higher log iPTH (P = 0.0001), and were receiving higher doses of intravenous VDAs (P = 0.0185) compared to those not taking cinacalcet.

**Discussion**

FGF-23 is an important regulator of mineral metabolism and plays an important role in CKD-MBD. In this study, cinacalcet use was independently associated with higher log FGF-23 in hemodialysis patients. Data from both animal and human studies suggest that cinacalcet therapy may in fact reduce circulating FGF-23. However, it remains unclear if cinacalcet directly affects FGF-23 or indirectly regulates FGF-23 via changes in
other clinical parameters of mineral and bone disorder. Uremic rats treated for 6 weeks with 15 mg/kg of cinacalcet had lower serum levels of FGF-23 levels compared to controls. Of note, the reduction in circulating FGF-23 concentrations observed in cinacalcet treated rats was accompanied by a simultaneous decrease in 1,25-dihydroxyvitmain D, a known stimulus of FGF-23 production. In addition, a recent open-label, single arm clinical trial conducted in hemodialysis patients with SHPT demonstrated that cinacalcet lowered FGF-23 levels, independent of VDAs. However, concomitant reductions in serum calcium, phosphorus and PTH were also observed. Thus, our results should be taken in the context of these previous published studies. It is possible that the observed relationship between FGF-23 and cinacalcet use in our study may be attributed to other factors present in the cinacalcet group known to alter FGF-23 such as serum phosphorus, activated vitamin D or PTH reduction.

In patients with CKD, higher phosphorus exposure has been associated with increases in FGF-23 concentrations. Whereas reducing phosphate load, either by dietary phosphorus restriction or use phosphate binding agents lowers FGF-23 levels. Similar to other published data, a positive independent association between serum phosphorus levels and FGF-23 was observed in this dialysis population. However, serum phosphate concentrations were similar in patients treated with cinacalcet compared to those who were not, suggesting that the observed relationship between cinacalcet use and FGF-23 was not due to differences in phosphate control. In addition, recent data indicates that non-calcium based binders reduce FGF-23 more substantially compared to calcium based phosphate binders. A post-hoc analysis of the BRIC study demonstrated that use of sevelamer for 1 year resulted in a significant decrease in FGF-23 from baseline in
hemodialysis patients, whereas a non-significant decrease was observed in patients treated with calcium acetate.\textsuperscript{24} These results are similar to a study in pre-dialysis CKD patients which found that short-term treatment with sevelamer resulted in a significant decrease in FGF-23.\textsuperscript{34} Yet, our study failed to show a relationship between phosphate binder class (i.e. non-calcium based versus calcium based) and FGF-23. This may be due to the lack of a controlled setting.

1,25-dihydroxyvitamin D directly stimulates FGF-23 expression in osteocytes.\textsuperscript{21} In ESRD, several clinical studies have illustrated that activated vitamin D administration is associated with subsequent elevations in FGF-23 levels,\textsuperscript{22-25} possibly in a dose dependent manner.\textsuperscript{25} A secondary analysis of the ACHIEVE trial showed that treatment with cinacalcet plus fixed low-dose VDAs (Cinacalcet-D) compared to the use of titrated VDAs alone (Flex-D) resulted in significantly lower FGF-23 levels after 27 weeks of treatment.\textsuperscript{25} Subjects in the Flex-D group received roughly 2.5 times the average weekly dose of VDA therapy compared to the Cinacalcet-D patients.\textsuperscript{25} Essentially, the use of titratable cinacalcet in combination with fixed low-dose VDAs allowed for a VDA sparing effect while providing similar PTH control, accounting for the lower FGF-23 levels in Cinacalcet-D group. In our study population, unlike the ACHIEVE trial, patients taking cinacalcet were receiving larger doses of VDAs. Thus, the observed relationship between cinacalcet use and FGF-23 concentration may be related to the higher level of activated vitamin D exposure among those taking cinacalcet. It is possible that the stimulatory effects of VDAs on FGF-23 outweighed the suppressive effects of cinacalcet in our patient population.
PTH acts on bone stimulating FGF-23 expression. Numerous clinical investigations have demonstrated strong correlations between PTH and FGF-23, similar to our results. In dialysis patients with advanced SHPT, surgical ablation of parathyroid glands result in significant reductions in serum FGF-23 along with concomitant decreases in PTH and phosphorus. In hemodialysis patients, elevated FGF-23 concentrations are indicative of parathyroid gland hyperplasia and have been a predictor for the development of refractory SHPT. Typically, VDAs are first line agents for the treatment of SHPT. Often, cinacalcet therapy is added on, in cases of severe disease, when further PTH reduction is required. In our dialysis population, subjects taking cinacalcet had higher PTH levels and were receiving larger doses of VDAs despite use of 51.5 ± 41.3 mg/day of cinacalcet for an average of 6.9 ± 6.3 months. These results suggest that the observed association between cinacalcet and FGF-23 is possibly a function of SHPT disease severity and leaves to question the ability of cinacalcet to lower FGF-23 in the setting of severe SHPT (i.e. patients with parathyroid gland hyperplasia).

We acknowledge that there are limitations to this study. The small sample size and the cross-sectional study design make it difficult to draw definitive conclusions regarding the true cause effect relationship between clinical parameters of CKD-MBD, related medication use and FGF-23. Typical treatment of CKD-MBD in the dialysis population often involves multi-drug medication regimens making the evaluation of a single drug effects difficult in observational studies. Additionally, differences in practice patterns in regards to initiation of cinacalcet therapy, intravenous VDA dosing algorithms
and phosphate binder selection may lead to different associations in other hemodialysis populations.

In summary, the observed association between cinacalcet use and FGF-23 appeared to be a function of SHPT disease severity, as indicated by the presence of higher PTH levels and the use of larger doses of activated vitamin D among patients on cinacalcet therapy. Results from other published trials regarding the effect of MBD therapies on FGF-23 indicate that FGF-23 is a key player in CKD-MBD. Given that elevated FGF-23 concentrations have been independently associated with mortality in the dialysis population,\textsuperscript{19,20} FGF-23 may become a therapeutic target in the near future. Needless to say, the optimal FGF-23 level and the true clinical significance of reducing FGF-23 with CKD-MBD medications remains unknown and needs to be addressed.
TABLES

Table 2. Subject characteristics$^a$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Subjects (n = 118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.5 ± 16.3</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>68 (58)</td>
</tr>
<tr>
<td>Race [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>100 (85)</td>
</tr>
<tr>
<td>African American</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Etiology of ESRD [n (%)]</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>51 (43)</td>
</tr>
<tr>
<td>HTN</td>
<td>43 (36)</td>
</tr>
<tr>
<td>DM and HTN</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Other</td>
<td>20 (17)</td>
</tr>
<tr>
<td>Dialysis vintage (years)</td>
<td>3.3 ± 2.9</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>iPPTH (pg/ml)</td>
<td>438.6 ± 499.5</td>
</tr>
<tr>
<td>Log iPPTH (pg/ml)</td>
<td>2.47 ± 0.41</td>
</tr>
<tr>
<td>Corrected calcium (mg/dl)</td>
<td>9.0 ± 0.6</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.5 ± 1.8</td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>31.9 ± 15.9</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.7 ± 1.4</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>469.6 ± 312.5</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>28.4 ± 9.9</td>
</tr>
<tr>
<td>FGF-23(pg/ml)</td>
<td>491.0 ± 684.5</td>
</tr>
<tr>
<td>Log FGF-23 (pg/ml)</td>
<td>2.50 ± 0.36</td>
</tr>
</tbody>
</table>
Data are expressed as mean ± standard deviation. 25(OH)D, 25-hydroxyvitamin D; DM, diabetes mellitus; ESRD, end-stage renal disease; FGF-23, fibroblast growth factor 23; GN, glomerulonephritis; HTN, hypertension; iPTH, intact parathyroid hormone; TSAT, transferrin saturation.
Table 3. Multiple linear regression model showing the association of log FGF-23 concentrations with clinical parameters and medication treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>β Estimate</th>
<th>Standard error</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model intercept</td>
<td>0.0337</td>
<td>0.3688</td>
<td>-0.6970 to 0.7644</td>
<td>0.9271</td>
</tr>
<tr>
<td>Cinacalcet use</td>
<td>0.1440</td>
<td>0.0506</td>
<td>0.0438 to 0.2442</td>
<td>0.0052</td>
</tr>
<tr>
<td>Dialysis vintage</td>
<td>0.0266</td>
<td>0.0081</td>
<td>0.0106 to 0.04262</td>
<td>0.0013</td>
</tr>
<tr>
<td>Corrected calcium</td>
<td>0.1926</td>
<td>0.0391</td>
<td>0.1151 to 0.2700</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.1101</td>
<td>0.0129</td>
<td>0.0844 to 0.1358</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Table 4. Descriptive summary of patient variables, laboratory parameters and relevant medication use between subjects using cinacalcet and those not on cinacalcet therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cinacalcet (n = 38)</th>
<th>No cinacalcet (n = 80)</th>
<th>P^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.8 ± 16.8</td>
<td>66.2 ± 15.5</td>
<td>0.0085</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>18 (47)</td>
<td>50 (63)</td>
<td>0.1201</td>
</tr>
<tr>
<td>Race [n (%)]</td>
<td></td>
<td></td>
<td>0.0328</td>
</tr>
<tr>
<td>Caucasian</td>
<td>29 (76)</td>
<td>71 (89)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>6 (16)</td>
<td>9 (11)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Etiology of ESRD [n (%)]</td>
<td></td>
<td></td>
<td>0.7559</td>
</tr>
<tr>
<td>DM</td>
<td>14 (37)</td>
<td>37 (46)</td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>16 (42)</td>
<td>27 (34)</td>
<td></td>
</tr>
<tr>
<td>DM and HTN</td>
<td>1 (3)</td>
<td>3 (4)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (18)</td>
<td>13 (16)</td>
<td></td>
</tr>
<tr>
<td>Dialysis vintage (years)</td>
<td>3.7 ± 2.9</td>
<td>3.1 ± 2.9</td>
<td>0.2537</td>
</tr>
<tr>
<td>FGF-23 (pg/ml)</td>
<td>722.6 ± 992.1</td>
<td>381.0 ± 441.6</td>
<td>0.0485</td>
</tr>
<tr>
<td>Log FGF-23 (pg/ml)</td>
<td>2.63 ± 0.41</td>
<td>2.44 ± 0.31</td>
<td>0.0185</td>
</tr>
<tr>
<td>iPTH</td>
<td>680.0 ± 744</td>
<td>324 ± 261.9</td>
<td>0.0065</td>
</tr>
<tr>
<td>Log iPTH (pg/ml)</td>
<td>2.68 ± 0.36</td>
<td>2.37 ± 0.40</td>
<td>0.0001</td>
</tr>
<tr>
<td>Corrected calcium (mg/dl)</td>
<td>8.9 ± 0.6</td>
<td>9.0 ± 0.6</td>
<td>0.6740</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.7 ± 1.8</td>
<td>5.4 ± 1.9</td>
<td>0.4443</td>
</tr>
<tr>
<td>Measure</td>
<td>Group A Mean ± SD</td>
<td>Group B Mean ± SD</td>
<td>P Value</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>---------</td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>33.7 ± 14.1</td>
<td>31.0 ± 16.7</td>
<td>0.3929</td>
</tr>
<tr>
<td>IV VDA use [n (%)]</td>
<td>30 (79)</td>
<td>53 (66)</td>
<td>0.1583</td>
</tr>
<tr>
<td>IV VDA dose (mcg/week as doxercalciferol-equivalents)</td>
<td>14.3 ± 11.2</td>
<td>9.6 ± 5.9</td>
<td>0.0405</td>
</tr>
<tr>
<td>Ergocalciferol use [n (%)]</td>
<td>18 (47)</td>
<td>24 (30)</td>
<td>0.0656</td>
</tr>
<tr>
<td>Phosphate binder use [n (%)]</td>
<td>33 (87)</td>
<td>67 (84)</td>
<td>0.6625</td>
</tr>
</tbody>
</table>

aData are expressed as mean ± standard deviation. 25(OH)D, 25-hydroxyvitamin D; DM, diabetes mellitus; ESRD, end-stage renal disease; FGF-23, fibroblast growth factor 23; GN, glomerulonephritis; HTN, hypertension; iPTH, intact parathyroid hormone; IV VDA, intravenous vitamin D analog.

bP values are for comparisons between groups (cinacalcet versus no cinacalcet) obtained using Student’s t-Test, χ² test and Fisher’s exact test where appropriate.
CHAPTER 3: CONCLUDING CHAPTER

Significance of Research to the Field and Potential for Future Research

FGF-23 has garnered much attention by the nephrology community and is becoming a leading candidate as a future therapeutic target in CKD-MBD. Higher circulating concentrations of FGF-23 have been associated with cardiovascular events\textsuperscript{18} and mortality.\textsuperscript{19, 20} However, the optimal level of FGF-23 and clinical impact of lowering FGF-23 in the hemodialysis population remains unknown; but is an emerging area of research.

Mineral metabolic parameters and medications used in the treatment of CKD-MBD affect the circulation concentrations of FGF-23. The purpose of this study was to describe the relationship between clinical parameters of CKD-MBD, related medications and FGF-23 in the hemodialysis population. Given the multifaceted nature of FGF-23 regulation, exploratory studies, such as this, are needed to understand how current medication therapy regimens and clinical practices are related to FGF-23 concentrations to provide evidence for the conduct of more extensive observational and interventional studies.

The most interesting finding of this study was the positive independent association between log FGF-23 and cinacalcet usage. Current guidelines recommend activated vitamin D agents as first line therapy for the treatment of SHPT.\textsuperscript{2} In cases of more severe disease, when use of vitamin D therapy alone cannot control PTH levels, cinacalcet therapy is added on. A reduction in PTH levels can be seen within three weeks of initiating cinacalcet.\textsuperscript{47} Unfortunately, some patients will continue to have marked elevations in serum parathyroid hormone levels despite the use of both vitamin D analogs
and cinacalcet; these patients are classified as treatment refractory. In this sub-set of patients hyperfunctioning parathyroid tissue does not respond appropriately to physiological regulation or to medical therapy due to intrinsic gland factors, decreased levels of vitamin D and calcium sensor receptors.\(^{48}\)

FGF-23 mediates PTH secretion indirectly, through its effects on phosphorus excretion and vitamin D metabolism and directly acts on the parathyroid gland to decrease PTH synthesis and secretion.\(^5,\)\(^6\) Often, in dialysis patients, PTH levels remain elevated despite exceptionally high FGF-23 concentrations indicating resistance of the parathyroid gland to FGF-23 in this setting.\(^{23,42,43}\) In the clinical setting, FGF-23 levels are a predictor for the development of treatment refractory SHPT,\(^{43}\) higher concentrations were associated with the future development of resistant hyperparathyroidism. In this study, patients taking cinacalcet had higher levels of PTH despite the use of activated vitamin D, use of cinacalcet for nearly 7 months and extremely elevated FGF-23 concentrations possibly suggest that this sub-set of patients had refractory hyperparathyroidism. However, the cross-sectional nature of this investigation makes it impossible to definitely conclude that the observed relationship between cinacalcet and FGF-23 was due to the presence of treatment resistant SHPT in those receiving cinacalcet given that prior PTH levels and past response to therapy were unknown. This further reaching explanation of the observed results is solely noted due to its hypothesis generating potential.

The results of this investigation taken in context with other published literature indicate that multiple CKD-MBD parameters (i.e. serum phosphorus, serum calcium, PTH) and medication use impact FGF-23 concentrations. Future research in the
hemodialysis population should aim to: 1) assess the clinical impact of lowering FGF-23 with existing CKD-MBD therapies; 2) determine the optimal FGF-23 level in hemodialysis patients; and 3) establish if cinacalcet therapy can lower FGF-23 in the settings of severe and treatment refractory SHPT.
### Additional Tables

**Table 5. Bivariate analysis of categorical variables to assess relationships with log FGF-23**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
<th>Log FGF-23 (pg/mL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>68 (57)</td>
<td>2.51 ± 0.36</td>
<td>0.7493</td>
</tr>
<tr>
<td>Female</td>
<td>50 (43)</td>
<td>2.49 ± 0.36</td>
<td></td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>100 (85)</td>
<td>2.51 ± 0.35</td>
<td>0.3456</td>
</tr>
<tr>
<td>Other</td>
<td>18 (15)</td>
<td>2.43 ± 0.37</td>
<td></td>
</tr>
<tr>
<td><strong>IV VDA use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>83 (70)</td>
<td>2.49 ± 0.32</td>
<td>0.7461</td>
</tr>
<tr>
<td>No</td>
<td>35 (30)</td>
<td>2.52 ± 0.42</td>
<td></td>
</tr>
<tr>
<td><strong>Ergocalciferol use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>42 (36)</td>
<td>2.51 ± 0.39</td>
<td>0.9119</td>
</tr>
<tr>
<td>No</td>
<td>76 (64)</td>
<td>2.50 ± 0.34</td>
<td></td>
</tr>
<tr>
<td><strong>Cinacalcet use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38 (32)</td>
<td>2.63 ± 0.41</td>
<td>0.0185</td>
</tr>
<tr>
<td>No</td>
<td>80 (68)</td>
<td>2.44 ± 0.31</td>
<td></td>
</tr>
</tbody>
</table>

*P values are for comparisons between groups obtained using Student’s t-Test, or the Wilcoxon rank-sum test where appropriate*
Table 6. Bivariate analysis of continuous variables to assess relationships with log FGF-23

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Log FGF-23 (pg/mL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>118</td>
<td>-0.2747</td>
<td>0.0026</td>
</tr>
<tr>
<td>Dialysis vintage (years)</td>
<td>118</td>
<td>-0.2623</td>
<td>0.0041</td>
</tr>
<tr>
<td>Log PTH (pg/mL)</td>
<td>118</td>
<td>0.3100</td>
<td>0.006</td>
</tr>
<tr>
<td>Corrected calcium (mg/dL)</td>
<td>118</td>
<td>0.2500</td>
<td>0.0063</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>118</td>
<td>0.5601</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (ng/mL)</td>
<td>115</td>
<td>-0.0917</td>
<td>0.3296</td>
</tr>
<tr>
<td>IV VDA dose (mcg/week as doxercalciferol-equivalents)</td>
<td>118</td>
<td>0.0099</td>
<td>0.9155</td>
</tr>
</tbody>
</table>

*The linear relationship between log FGF-23 and each normally distributed continuous parameter were evaluated using Pearson’s correlation*
REFERENCES


APPENDICES
Appendix A: Research Ethics

Given the importance of ethics in the conduct of scientific research, critical ethical considerations that were assessed in relation to this study are outlined below.

Institutional Review Board (IRB) Exemption

In order to conduct this study, pertinent de-identified data needed to be extracted from an existing de-identified database (owned by my fellowship mentor Dr. Darius L. Mason), for each patient. Thus, this project meets exemption category 4 of 45 CFR 46.101(b)(4): “research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.” As a result, the protocol was granted an IRB Exemption by the Albany College of Pharmacy and Health Sciences IRB.

Waiver of Consent

Given that this protocol was submitted for IRB exempt review, a waiver of consent was also requested by the investigators and was granted. This study met the conditions for a waiver of consent, as described in 45 CFR 46.116d. The conditions for a waiver of consent and how this project met each of these conditions is described below:

(1) the research involves no more than minimal risk to the subjects;

All data utilized in this study was collected from an existing de-identified database and analyzed retrospectively. Thus, there is no risk was posed to the subjects.
(2) the waiver will not adversely affect the rights and welfare of the subjects; All data utilized in this study was be collected from an existing de-identified database and analyzed retrospectively. Thus, this study did not affect the rights and welfare of the patients.

Confidentiality

All of the data to be analyzed in this study was part of a pre-existing de-identified database. The pertinent variables from the VDR PGX database were extracted and entered into a new electronic database using the patients’ previously assigned unique identification numbers. The database created for the purpose of this investigation was password protected and stored on a password protected computer in the locked office of the principal investigator. The electronic database will be maintained indefinitely by the principal investigator. Thus, ensuring that confidentiality was maintained throughout the conduct of the study.

Risk Benefit Ratio

Documentation of Risk

This study poses no risk to patient due to the fact that it was an analysis of a pre-existing de-identified dataset.

Benefits

The results of this study enhanced the general understanding of the relationship between clinical parameters of CKD-MBD, FGF-23 and related medication use in the hemodialysis population. Finding the optimal CKD-MBD therapy regimen which controls PTH, phosphorus, calcium, and FGF-23 may have clinical significance in the dialysis population.
Appendix B: IRB Exemption

February 28, 2011

Magdalene Assimon, Pharm.D.
Nephrology Pharmacotherapy Research Fellow
Department of Pharmacy Practice
Albany College of Pharmacy and Health Sciences
106 New Scotland Avenue
Albany, NY 12208

Protocol #: 11-001
Title: Evaluation of the Relationship between Clinical Parameters of Mineral and Bone Disease, Fibroblast Growth Factor-23, and Related Medication Use among Hemodialysis Patients

Dear Dr. Assimon:

This letter is to notify you that the Institutional Review Board of Albany College of Pharmacy and Health Sciences (IRB) protocol referenced above was received and approved as exempt from IRB review.

The protocol met the requirements of exempt category 4 of 45 CFR 46.101(b). The protocol sufficiently demonstrated the proposed research involves the collection or study of existing data, documents, records ..., (and) ... the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

The requirement to obtain informed consent was waived as the following criteria were met 45 CFR 46.116(d):

- the research involves no more than minimal risk to the subjects;
- the waiver or alteration will not adversely affect the rights and welfare of the subjects;
- the research could not practicably be carried out without the waiver or alteration; and
- if/whenever appropriate, the subjects will be provided with additional pertinent information after participation.

No final or continuing report is required; however, any changes in research activity must be promptly reported to the IRB.

You should reference your protocol number whenever corresponding with the IRB, whether by mail or in person. If you have questions, please contact me.

Sincerely,

Sunita M. Chowfin
IRB Administrator

cc: File