Dose response of PTH and FGF23 to Paricalcitol in patients with end stage renal failure on chronic intermittent haemodialysis

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DOSE RESPONSE OF PTH AND FGF23 TO PARICALCITOL IN PATIENTS WITH END STAGE RENAL FAILURE ON CHRONIC INTERMITTENT HAEMODIALYSIS

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1. INTRODUCTION

1.1 Definition

Chronic kidney disease (CKD) is characterized by the presence of kidney damage or reduced kidney function lasting for a minimum of three months, regardless of the underlying cause. Kidney damage is typically identified by pathologic abnormalities in the native or transplanted kidneys, which can be confirmed through imaging, biopsy, or inferred from clinical markers. These markers include elevated albuminuria, indicated by an albumin-to-creatinine ratio (ACR) greater than 30 mg/g (3.4 mg/mmol), or changes in urinary sediment. Reduced kidney function is determined by a lower glomerular filtration rate (GFR), which is usually estimated (eGFR) from the serum creatinine concentration (1). CKD can also be present in patients with an eGFR of 60 ml/min or higher (CKD stage I and II) if other signs of kidney damage are detected (2). Progressive and significant deterioration in kidney function can lead to end-stage renal failure, necessitating renal replacement therapy (e.g., dialysis or transplantation), depending on the extent of kidney damage (3).

Chronic kidney disease is categorized into six stages based on the GFR in connection with detectable kidney damage (3):

- G1: GFR 90 ml/min per 1.73 m^2 body surface area and above
- G2: GFR 60 to 89 ml/min per 1.73 m^2
- G3a: GFR 45 to 59 ml/min per 1.73 m^2
- G3b: GFR 30 to 44 ml/min per 1.73 m^2
- G4: GFR 15 to 29 ml/min per 1.73 m^2
- G5: GFR less than 15 ml/min per 1.73 m2 or treatment by dialysis

Additionally, albuminuria is classified into three levels based on the albumin-creatinine ratio (ACR) (3):

- A1: ACR less than 30 mg/gm (less than 3.4 mg/mmol)
- A2: ACR 30 to 299 mg/gm (3.4 to 34 mg/mmol)
- A3: ACR greater than 300 mg/gm (greater than 34 mg/mmol).

The combination of these two parameters is used to predict the renal and, extending beyond this, also general prognosis.

1.2 Epidemiology

Chronic kidney disease (CKD) is a progressive disorder that affects around 10% of the global population, impacting over 800 million people worldwide. The prevalence of CKD increases with age and is more common in women, racial minorities, and individuals with diabetes mellitus and hypertension. The burden of CKD is exceptionally high in poorer countries, which often lack the resources to manage its consequences effectively (4).

CKD is a leading cause of death globally and is one of the few non-communicable diseases that have seen a rise in mortality rates over the past two decades. In 2017, CKD affected an estimated 843.6 million people globally. The Global Burden of Disease studies highlight the significant rising in CKD as a cause of death, necessitating improved prevention and treatment efforts (4).

The prevalence of CKD varies significantly by age, with higher rates observed in older populations. For instance, CKD stages 1–4 affect 5.6% of individuals aged 20 to 39 years, while the rate rises to 44% among those over 70 years old. This age-related increase in prevalence is likely due to the natural decline in glomerular filtration rate (GFR) with age (4).

Gender differences in CKD prevalence have been observed, with women showing higher rates than men. The prevalence of CKD stages 1–4, adjusted for age, was found to be 14.9% in women and 12.3% in men in the United States. These differences may be partly due to the CKD definition, which uses a single GFR cutoff that might overdiagnose CKD in women. The progression of CKD tends to be more rapid in men than in women (4).

Differences in CKD prevalence across racial groups are well-recognized, particularly in the United States. For instance, age-adjusted prevalence rates for CKD stages 1–4 are 13% among non-Hispanic Whites, 16.5% among non-Hispanic Blacks, and 15.3% among Mexican Americans. These disparities are largely due to varying rates of CKD risk factors, including diabetes mellitus, hypertension, obesity, genetic predispositions, lifestyle differences, and socioeconomic inequalities (4). The most common risk factors for CKD include diabetes, hypertension, obesity, older age, substance use (such as smoking, alcohol, and recreational drugs), a family history of CKD, and being of non-Hispanic Black or Hispanic descent (5).

Diabetes mellitus and hypertension are the primary risk factors for the development of CKD. In the United States, between 2011 and 2014, CKD stages 3–4 were found in 24.5% of individuals diagnosed with diabetes. In comparison, the prevalence of CKD in those with prediabetes was 14.3%, and in those without diabetes, it was 4.9%. Similarly, CKD prevalence

among adults with hypertension was 35.8%, while it was 14.4% in those with prehypertension and 10.2% in individuals without hypertension (4).

Secondary hyperparathyroidism (sHPT) is a frequent complication in CKD, particularly in advanced stages. It results from the kidneys' inability to maintain calcium and phosphate balance, leading to increased parathyroid hormone (PTH) secretion. The prevalence of sHPT increases with the severity of CKD. sHPT contributes significantly to the morbidity and mortality of CKD patients due to its association with bone disease and cardiovascular complications (6,7).

In conclusion, CKD represents a significant global public health challenge, with its prevalence on the rise and significant mortality risk. The disease's burden is disproportionately higher in older adults, women, racial minorities, and individuals with comorbid conditions such as diabetes and hypertension. Effective prevention and management strategies are crucial to address this growing health concern including pathological disturbances in hormonal and electrolyte homeostasis.

1.3 Etiology

Chronic kidney disease can arise from any condition that impacts the kidney's structure or function. The etiology of CKD varies globally, with several primary diseases contributing to its development and progression to end-stage renal disease (ESRD) (3).
Some of the known causes of CKD (incidence in brackets) are (3):

- Diabetes mellitus type 2 (30% to 50%)
- Diabetes mellitus type 1 (3.9%)
- Hypertension (27.2%)
- Primary glomerulonephritis (8.2%)
- Chronic tubulointerstitial nephritis (3.6%)
- Hereditary or cystic diseases (3.1%)
- Secondary glomerulonephritis or vasculitis (2.1%)
- Plasma cell dyscrasias or neoplasm (2.1%)
- Sickle cell nephropathy (SCN) which accounts for less than 1% of ESRD patients in the United States

These causes can be divided into three primary categories: prerenal, intrinsic renal, and postrenal. Chronic prerenal disease typically affects patients with chronic heart failure or liver cirrhosis, resulting in a sustained reduction in renal blood flow. The likelihood of recurrent intrinsic kidney injury, such as acute tubular necrosis (ATN), is increased by this ongoing reduction in perfusion, which over time leads to a gradual deterioration in kidney function (3).

Intrinsic renal causes involve diseases affecting the blood vessels, glomeruli, or tubules-interstitium within the kidneys. Nephrosclerosis is the most prevalent chronic renal vascular disease, leading to sustained damage to the blood vessels, glomeruli, and tubulointerstitium. Renal artery stenosis, which can arise from atherosclerosis or fibromuscular dysplasia, is another vascular condition that can lead to ischemic nephropathy. Over time, this condition may progress to glomerulosclerosis and tubulointerstitial fibrosis. Intrinsic glomerular diseases may manifest in either nephritic or nephrotic patterns. A nephritic pattern typically involves findings such as red blood cell casts, dysmorphic red cells, white blood cells, and variable proteinuria in urine microscopy. Common causes include post-streptococcal glomerulonephritis (GN), infective endocarditis, shunt nephritis, IgA nephropathy, lupus nephritis, Goodpasture syndrome, and vasculitis. In contrast, a nephrotic pattern is marked by significant proteinuria (exceeding 3.5 grams per 24 hours) and a relatively inactive urine microscopic analysis. Diseases like minimal change disease, focal segmental glomerulosclerosis, membranous GN, membranoproliferative GN (Types 1 and 2), diabetic nephropathy, and amyloidosis are common causes of this pattern. Polycystic kidney disease (PKD) is the most frequently encountered chronic tubulointerstitial disease. Other contributing factors include nephrocalcinosis, often resulting from hypercalcemia and hypercalciuria, sarcoidosis, Sjogren syndrome, and reflux nephropathy in both children and young adults. Additionally, CKD of unknown etiology, such as Mesoamerican nephropathy, is increasingly being recognized among agricultural workers in Central America and parts of Southeast Asia (3).

CKD can have postrenal causes, such as persistent obstruction from prostatic disease, nephrolithiasis, or abdominal/pelvic tumors that exert mass effects on the ureter(s) (3).

In summary, CKD results from a variety of etiologies, each with distinct pathophysiological mechanisms. Understanding these underlying causes is crucial for effectively diagnosing, managing, and preventing CKD progression to ESRD.

1.4 Pathophysiology

The pathophysiology of chronic kidney disease (CKD) is highly complex and remains an active area of research. It varies depending on the underlying cause, but all forms of CKD ultimately lead to progressive nephron loss, structural damage, and impaired kidney function (8). Below, I will highlight three of the most significant underlying conditions, their mechanisms, and their respective consequences.

Underlying conditions include diabetic nephropathy, hypertensive nephropathy, and glomerulonephritis (8).

Diabetic Nephropathy: This is the most common cause of CKD (3). The primary mechanism of damage involves hyperglycemia leading to nonenzymatic glycation of proteins, causing various degrees of harm on different types of kidney cells. These changes manifest as hypertrophy and proliferation of mesangial cells, glomerular basement membrane (GBM) thickening, and extracellular matrix (ECM) protein accumulation, resulting in eosinophilic nodular glomerulosclerosis. Additional changes include thickening and widespread hyalinization of afferent and efferent arterioles/interlobular arteries, interstitial fibrosis, tubular basement membrane (TBM) thickening, and tubular hypertrophy (8,9).

Hypertensive Nephropathy: This is the second most common cause of CKD and results from autoregulatory vasoconstriction of preglomerular vessels. Typically, limited increases in systemic blood pressure do not affect renal microvessels. However, chronic hypertension can push blood pressure beyond the protective autoregulatory limits, triggering sclerosis of afferent arterioles and small arteries (benign nephrosclerosis), decreased perfusion, and ischemic damage (8,10,11). Acute injury due to excessively high blood pressure can lead to malignant nephrosclerosis, characterized by petechial subcapsular hemorrhages, visible infarction with necrosis of mesangial and endothelial cells, thrombosis of glomerular capillaries, luminal thrombosis of arterioles, and red blood cell extravasation and fragmentation (8,12).

Glomerulonephritis (GN): This is the third most common cause of CKD and can be subdivided into nephrotic GN (e.g., minimal change disease, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis) and nephritic GN (e.g., IgA nephropathy, Henoch Schonlein purpura, post-streptococcal GN) (3,13).

The reduction in GFR leads to multiple downstream effects. Decreased GFR results in less urine production, increasing extracellular fluid volume and total-body volume overload (8,14). There is also less excretion of waste products, such as urea and drugs, causing their accumulation (8). Reduced phosphate excretion causes hyperphosphatemia (8,15).

Hyperphosphatemia and hypocalcemia trigger an increase in PTH (see following paragraph), and while fibroblast growth factor 23 (FGF-23) also helps stabilize plasma phosphate levels in the early stages of CKD, its effects diminish in advanced stages due to the development of resistance in target tissues (6,8,16). Additionally, reduced kidney function impairs acid-base balance, leading to metabolic acidosis as the kidneys can no longer excrete organic acids effectively. There is less maintenance of electrolyte concentrations, resulting in imbalances such as sodium (Na^+) retention (3,8).

As an endocrine organ, the kidneys adapt and respond differently or less to stimuli in CKD. Reduced erythropoietin secretion by peritubular interstitial cells leads to decreased stimulation of erythropoiesis (3,8). Additionally, decreased hydroxylation of calcifediol and production of calcitriol, combined with reduced phosphate excretion, lower serum calcium (Ca^{2+}) levels and increase parathyroid hormone (PTH) levels (6,8,17). Impaired kidney function also affects gluconeogenesis, increasing the risk of hypoglycemia (8,18). The question of defining physiological adaption and pathophysiological maladaption takes center stage to our understanding, and, hence, to successful treatment.

1.5 Clinical presentation

Many patients with chronic kidney disease (CKD) are asymptomatic in the early stages (until stages IV and V) due to the compensatory mechanisms of the kidney (3,8). Most clinical features manifest either due to sodium/water (Na^+/H_2O) retention or uremia (8).

Na^+/H_2O retention can lead to hypertension, heart failure, pulmonary edema, usually interstitial pulmonary edema, and peripheral edema (8).

The accumulation of toxic substances due to decreased renal excretion is termed uremia. These substances are primarily metabolites of proteins, such as urea, creatinine, α-macroglobulin, β2-macroglobulin, and PTH. Common symptoms include (8,15):

- Constitutional symptoms
  - Fatigue
  - Muscle weakness
  - Headaches
- Gastrointestinal symptoms
  - Nausea and vomiting
  - Anorexia

        - Uremic fetor: A urine-like odor of the breath
    - Dermatological manifestations
        - Pruritus
        - Skin color changes
        - Uremic frost: Whitish urea crystals deposited on the skin
        - Xerosis
    - Serositis
        - Uremic pericarditis
    - Neurological symptoms
        - Asterixis
        - Signs of Uremic Encephalopathy
            - Seizures
            - Somnolence
            - Coma
    - Hematologic symptoms
        - Anemia
        - Increased bleeding tendency

1.6 Diagnosis

Chronic kidney disease (CKD) diagnosis requires evidence of kidney damage for ≥ 3 months (19). This can include irregularities detected in blood or urine tests, or imaging studies, even if the glomerular filtration rate (GFR) is ≥ 60 mL/min/1.73 m² (2). CKD can also be diagnosed if the estimated GFR (eGFR) is < 60 mL/min/1.73 m² for three months or more, regardless of other signs of kidney damage. Additionally, a urine albumin-to-creatinine ratio (ACR) > 30 mg/g can also indicate CKD (19).

Diagnostic procedures include initial laboratory tests, such as assessments of renal function and urine samples, ultrasound of the kidneys and urinary tract, additional investigations to identify underlying causes (e.g., HbA1c, serology, serum protein electrophoresis), and renal biopsy. Serum markers such as creatinine and blood urea nitrogen (BUN) (alternatively, cystatin C) are elevated in CKD, while eGFR is reduced. eGFR can be determined using serum creatinine (preferred) via equations such as the CKD-EPI or MDRD equation, or using serum cystatin C (8,19).

Urine studies include increased spot urine albumin-to-creatinine ratio (UACR) or spot urine protein-to-creatinine ratio (UPCR), urine dipstick tests, and urine microscopy. Spot UACR is used to determine the albuminuria category for CKD staging, while UPCR can indicate nephrotic-range proteinuria. Urine microscopy can detect sediment, such as the presence of casts, while urine dipstick tests may reveal hematuria or proteinuria (3,8,19).

Hemostasis might be altered with an elevated bleeding time, normal PT, PTT and platelet count (3,8,20).

Ultrasound imaging is the primary diagnostic tool for CKD, which helps visualize kidney structure and establish etiology. Key ultrasound findings in CKD include kidneys that are small with reduced cortical thickness, increased echogenicity, and the presence of cysts or scarring. Findings such as ureteral or renal pelvic dilation could point to specific causes like obstructive nephropathy, while the presence of bilaterally enlarged kidneys with multiple cysts might indicate PKD (3,8).

Further investigations are conducted when clinical suspicion arises or when the primary cause of CKD remains unclear. Examples include diabetes, indicated by high HbA1c and fasting plasma glucose levels; glomerulonephritis (GN); multiple myeloma; renal artery stenosis, suggested by treatment-resistant hypertension and abnormal duplex ultrasonography of the renal arteries; and amyloidosis (8).

Renal biopsy is not routinely performed but can be valuable for cases with a rapid onset and unexplained decrease in eGFR or when confirmation of the underlying cause, such as glomerulonephritis (GN), is needed before initiating specific treatment. It should be avoided if the potential benefits do not outweigh the risks, if there is already an established diagnosis, or if it is unlikely to influence the therapy (8,19).

1.7 Treatment

Management of CKD typically involves a combination of nutritional management, pharmacological therapy, the effective management of comorbidities such as hypertension, hyperlipidemia, and diabetes and finally renal replacement therapy, i.e. dialysis and renal transplantation (8).

Referring to a nutritionist can help manage the various complications of CKD. Patients need to ensure appropriate fluid intake and avoid dehydration. Dietary recommendations for CKD include following the Mediterranean diet, which emphasizes fruits and vegetables to improve lipid profiles, weight, and blood pressure control (8). While a slight restriction in

protein intake is often recommended, excessive reduction can lead to malnutrition (21). For CKD stages G3-G5, approximately 0.6-0.8 g/kg/day of protein is considered acceptable to prevent malnutrition while avoiding excessive intake that could lead to obesity (22). Additionally, adjustments in electrolyte and phosphorus intake are necessary, as these can accumulate in CKD. Sodium intake should be restricted to less than 2.3 g/day to reduce blood pressure, improve volume control, and decrease proteinuria. It is crucial to focus on a balanced, healthy diet rich in natural foods to promote a healthy gut flora, which multivitamin supplements alone cannot achieve. However, in cases where dietary intake may be insufficient, particularly for vitamin D, multivitamin supplementation can be considered (8,21). Many medications are renally cleared, so doses must be adjusted based on the patient's eGFR. Nephrotoxic substances should be avoided (8,19). While the risk of contrast-agent induced nephropathy has significantly decreased with the use of modern iso-osmolar contrast agents (approximately 290 mosmol/l), careful consideration is still necessary for patients with eGFR < 30 mL/min/1.73 m² to avoid unnecessary exposure. The benefits of essential imaging should be weighed against potential risks, and preventive measures such as adequate hydration should be employed to minimize any remaining risk (8,23–25).

Furthermore, renal replacement therapy is divided into non-operative (hemodialysis or peritoneal dialysis) and operative (kidney transplant). Indications for non-operative renal replacement therapy include (8,23):

- Hemodynamic or metabolic complications refractory to medical therapy, such as:
  - Volume overload or hypertension
  - Metabolic acidosis
  - Hyperkaliemia
- Serositis: e.g., uremic pericarditis
- Symptoms of uremia: e.g., signs of encephalopathy
- Refractory deterioration in nutritional status despite optimal dietary interventions

A target systolic blood pressure (SBP) of <120 mmHg is advised. For patients with limited life expectancy, significant comorbid conditions, or symptomatic postural hypotension, a higher target SBP, such as <130-140 mmHg, might be more appropriate. Treatment includes pharmacological therapy and nonpharmacological management. Pharmacological therapy includes first-line treatment with RAAS inhibitors (ACEI or ARB) for their nephroprotective

effects and reduction of proteinuria, although risks may include hyperkalemia and an initial decline in GFR. Combination therapies should be considered if the patient has an initial SBP > 20 mmHg above the target or does not reach the target on monotherapy at the optimal dose. Second-line agents include loop diuretics or thiazide diuretics, calcium channel blockers, beta-blockers (usually for patients with cardiovascular comorbidities), and aldosterone receptor antagonists (usually for treatment-resistant hypertension). The recommended nonpharmacological management for all patients includes lifestyle changes such as weight loss (the most effective measure), diet, and exercise (8,26).

For lipid management, the goal is to reduce atherosclerotic cardiovascular disease (ASCVD) risk. Fasting lipid panels may show dyslipidemia, particularly increased triglycerides. Treatment is divided into pharmacological therapy and nonpharmacological management. Pharmacological therapy primarily involves statin therapy, which is indicated for the prevention and management of ASCVD. Typically started in all patients ≥ 50 years old and considered for patients 18-49 years old with concomitant diabetes mellitus or a 10-year ASCVD risk > 10%. Nonpharmacological management focuses on lifestyle modifications such as dietary changes and regular exercise (27,28).

This is crucial in diabetic nephropathy, as some medications need to be reduced or stopped due to declining eGFR. HbA1c may not accurately reflect glycemic control in patients with CKD and eGFR < 30 mL/min/1.73 m^2. Certain diabetic medications (e.g., SGLT-2 inhibitors and GLP-1 receptor agonists) can reduce CKD progression, urinary albumin excretion, and ASCVD events (29).

Screening for depression and facilitating strong social-emotional support are important aspects of a holistic approach to patient care (20).

1.8 Paricalcitol

As explained above, vitamin D (calciferol) is essential for the regulation of calcium and phosphate metabolism. Paricalcitol is a synthetic vitamin D analog and is related to the substance class of steroid hormones, specifically calciferols, with the active form of vitamin D being 1,25-dihydroxyvitamin D (1,25-$(OH)_2$ D_3, calcitriol). In patients with CKD, paricalcitol is often administered to address secondary hyperparathyroidism (31).

Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are the two primary available forms of Vitamin D. Ergocalciferol is found in mushrooms and fortified foods such as milk, breakfast cereals, and formula, as well as in yeast derived from ergosterol.

Cholecalciferol, on the other hand, is synthesized in the skin (stratum basale) when exposed to UV light and is also found in fortified foods, fatty fish liver, egg yolks, and some plants (32,33).

The synthesis of vitamin D begins in the liver, where cholesterol is converted to 7-dehydrocholesterol (provitamin D3) by the enzyme cholesterol dehydrogenase. Provitamin D3 is stored in the skin, where UV light cleaves it to form cholecalciferol. This cholecalciferol is then hydroxylated in the liver to become 25-hydroxyvitamin D (25-OH D_3, calcidiol). In the kidneys, 1α-hydroxylase further hydroxylates 25-hydroxyvitamin D to form the active 1,25-dihydroxyvitamin D (34).

Transport of the active form to target cells is facilitated by vitamin D-binding protein (DBP). The regulation of vitamin D synthesis is tightly controlled: decreased calcium, decreased phosphate, and increased parathyroid hormone (PTH) levels stimulate 1α-hydroxylase activity, thereby increasing 1,25-dihydroxyvitamin D biosynthesis. Conversely, increased calcium, increased phosphate, and elevated levels of 1,25-dihydroxyvitamin D exert feedback inhibition on 1α-hydroxylase activity, reducing its biosynthesis (31,34,35).

Vitamin D is essential for calcium and phosphate metabolism. It facilitates the absorption of calcium and phosphate in the intestines and enhances calcium reabsorption in the kidneys. At standard vitamin D levels, it stimulates bone mineralization and remodeling both indirectly by maintaining serum calcium and phosphate levels and directly by activating osteoblasts and promoting osteoclast differentiation (35,36). However, at high vitamin D levels, it promotes bone resorption (37).

In summary, vitamin D, the only vitamin that the human body can fully synthesize on its own, plays a crucial role in maintaining calcium and phosphate balance and ensures proper bone health. Paricalcitol promises stronger control of PTH whilst affecting less the calcium and phosphate balance (35,38).

1.9 Parathyroid Hormone (PTH) and Fibroblast Growth Factor 23 (FGF23)

1.9.1 Parathyroid Hormone (PTH)

Parathyroid hormone (PTH) is secreted by the parathyroid glands to increase serum calcium and decrease serum phosphate levels. It exerts its effects on the kidneys, gastrointestinal tract, and bones. In the kidneys, PTH increases calcium reabsorption in the distal tubule and decreases phosphate reabsorption in the proximal convoluted tubules. It also stimulates 1-alpha-hydroxylase synthesis, leading to increased production of vitamin D (calcitriol). In the gastrointestinal tract, PTH indirectly increases calcium absorption by

enhancing 1,25-dihydroxyvitamin D (calcitriol) synthesis in the kidneys. In bones, PTH promotes the release of calcium and phosphate by increasing the expression of RANKL on osteoblasts, which binds to RANK receptors on osteoclasts, thereby increasing osteoclast activity and bone resorption (39).

Stimulation of PTH secretion occurs due to a decrease in serum calcium, hyperphosphatemia, mild hypomagnesemia (e.g., due to chronic diarrhea, inflammatory bowel disease (IBD), loop diuretics, or alcohol), and low calcitriol levels. In contrast, inhibition of PTH secretion is triggered by an increase in serum calcium and marked hypomagnesemia (40).

PTH levels are a critical concern in CKD due to the development of secondary hyperparathyroidism (sHPT). sHPT is a common and potentially serious complication of CKD that arises from disturbances in mineral metabolism and endocrine function as kidney function declines. The pathophysiology of sHPT involves several key mechanisms. As kidney function deteriorates, phosphate excretion diminishes, leading to hyperphosphatemia. Elevated phosphate levels directly stimulate parathyroid gland activity and PTH secretion enhancing excretion of phosphate. The failing kidneys are inhibited and less capable of converting 25-hydroxyvitamin D to its active form, 1,25-dihydroxyvitamin D (calcitriol). Calcitriol is crucial for calcium absorption in the gut. Reduced levels of calcitriol lead to decreased calcium absorption, resulting in hypocalcemia, which in turn stimulates PTH secretion. Reduced renal synthesis of calcitriol leads to vitamin D deficiency. Vitamin D normally suppresses PTH production, so its deficiency contributes to increased PTH secretion. This vitamin D deficiency also exacerbates hypocalcemia, further driving PTH release. Phosphate itself has a direct stimulatory effect on the parathyroid glands (6). High phosphate levels increase the expression of PTH and promote parathyroid cell proliferation, leading to gland hyperplasia (41). As a further counter regulatory factor, FGF23 increases in CKD, reducing the synthesis of calcitriol and promoting phosphate excretion. However, in advanced CKD, resistance to FGF23 can develop, leading to ineffective phosphate excretion, thus weakening its beneficial properties regarding reduction of hyperphosphatemia and attenuation of secondary hyperparathyroidism (16,42).

The elevated PTH levels in sHPT have several adverse effects. Chronic, inadequate elevation of PTH leads to increased bone resorption and turnover, resulting in renal osteodystrophy, which encompasses a range of bone disorders, including osteitis fibrosa cystica, characterized by bone pain, skeletal deformities, and increased fracture risk (6). As a consequence of disturbed electrolyte homeostasis, elevated PTH levels are linked to vascular calcification, arterial stiffness, and left ventricular hypertrophy. These changes contribute to an

increased risk of cardiovascular events and mortality in CKD patients (42). High PTH levels can also lead to the deposition of calcium in soft tissues (i.e. soft tissue calcification) and also predisposition to calciphylaxis, a serious and often fatal condition characterized by primarily necrotic skin lesions, but in seldom cases also of calcifying microvascular arterial disease in other organs (43).

Managing PTH levels in CKD involves addressing the underlying disturbances in mineral metabolism. Treatments include phosphate binders to reduce phosphate absorption, vitamin D analogs to increase calcium absorption and suppress PTH secretion, and calcimimetics to enhance the sensitivity of the parathyroid glands to calcium, thereby reducing PTH production (6). Paricalcitol, a vitamin D analog used in this study, has shown effectiveness in lowering PTH levels, thereby mitigating some of the complications associated with sHPT in CKD patients.

1.9.2 Fibroblast Growth Factor 23 (FGF23)

FGF23 is a hormone secreted by osteocytes and osteoblasts in response to increased active vitamin D (calcitriol) and phosphate levels. It plays a crucial role in phosphate metabolism by increasing phosphate excretion and decreasing vitamin D activation. Specifically, FGF23 acts on the kidneys to reduce phosphate reabsorption, thus increasing its excretion, and it reduces the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (calcitriol), leading to lower levels of active vitamin D and consequently reduced intestinal absorption of calcium and phosphate (27,28). Thus, FGF23 is an essential factor in the electrolyte-osteal regulatory feedback loop in conjunction with PTH.

The activity of FGF23 is mediated through its interaction with the fibroblast growth factor receptors (FGFRs), particularly FGFR1. The co-receptor Klotho is essential for the high-affinity binding of FGF23 to FGFR1. Klotho, predominantly expressed in the kidneys and parathyroid glands, acts as a co-receptor, enhancing the binding affinity of FGF23 to FGFR1 and facilitating its signaling pathway (42).

In early-stage CKD, increased secretion of FGF23 leads to higher phosphate excretion and reduced conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, maintaining normal phosphate levels initially. In advanced CKD, target tissues develop resistance to FGF23, resulting in diminished effects and ultimately leading to hyperphosphatemia (16). However, FGF23 is also known to have several adverse effects on the cardiovascular system, particularly in CKD patients where its levels are significantly elevated.

Recent research continues to emphasize the significant role of FGF23 in cardiovascular health, particularly in patients with CKD. A study published in 2024 in the Journal of the American College of Cardiology highlights that even though the effects of FGF23 on cardiovascular outcomes, particularly left ventricular hypertrophy (LVH), may be considered 'minor' in some contexts, they remain a focal point in ongoing cardiovascular research. The study suggests that elevated FGF23 levels are independently associated with increased left ventricular mass and a higher risk of adverse cardiovascular events and mortality in CKD patients. This finding reinforces the importance of FGF23 as a key target in managing CKD-related cardiovascular risks (45). Thus, FGF23 remains at the center of cardiovascular research, highlighting its complex role in CKD and necessitating ongoing investigation into its therapeutic management.

Elevated FGF23 levels contribute to cardiovascular injury through several mechanisms. FGF23 directly influences vascular smooth muscle cells and promotes vascular calcification, a process that leads to stiffening of blood vessels and increased cardiovascular risk. FGF23 has been shown to directly stimulate hypertrophy of the left ventricle. It activates the FGF receptor 4 (FGFR4) on cardiomyocytes, leading to increased intracellular calcium levels and subsequently stimulates the calcineurin-NFAT signaling pathway, which promotes hypertrophic growth of cardiomyocytes (46). Elevated FGF23 levels are associated with increased systolic and diastolic blood pressures. FGF23 may affect sodium reabsorption in the kidneys, contributing to volume overload and hypertension. Additionally, FGF23 can act on the central nervous system to increase sympathetic nerve activity, further contributing to high blood pressure (42). Chronic elevation of FGF23 can lead to fibrosis of the cardiac tissue, impairing cardiac function and increasing the risk of heart failure. This fibrosis is mediated through FGF23's interaction with its receptors and subsequent activation of pro-fibrotic signaling pathways in cardiac fibroblasts (44). These mechanisms collectively contribute to the heightened cardiovascular risks and increased mortality rates observed in CKD patients with high FGF23 levels. Managing FGF23 levels through therapeutic interventions is crucial in mitigating these risks.

Current treatments for managing FGF23-related complications in CKD include calcimimetics, phosphate binders, and hemodiafiltration. Future treatments may involve blocking the FGF23 receptor or using agents like oleic acid to reduce LVH (6,46,47).

In conclusion, FGF-23 is primarily a phosphaturic hormone that induces left ventricular hypertrophy over the long term, increasing overall mortality risk.

Historically, administration of active vitamin D and paricalcitol with the intention of reducing PTH has been associated with an increase in FGF23 (48).

2. OBJECTIVES

2.1 Aim of the Analysis

The objective of this study was to determine the impact of paricalcitol on serum fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) levels in patients with CKD stage 5 on chronic intermittent haemodialysis in comparison with placebo.

Specifically, the study saught to:

1. Determine the effects of paricalcitol on PTH levels over a 12-week period.
2. Evaluate the impact of paricalcitol on FGF23 levels over the same period.
3. Assess the influence of paricalcitol dose reduction after six weeks on PTH and FGF23 levels.

2.2 Hypotheses

Primary Hypothesis:

Paricalcitol significantly reduces PTH levels over a 12-week period compared to placebo.

Secondary Hypotheses:

- Paricalcitol induces an increase in FGF23 levels at 6 weeks and 12 weeks.
- The effect of dose adjustment, i.e. reduction, in paricalcitol on FGF23 levels after 6 and 12 weeks treatment aiming primarily at achieving adequate PTH suppression, historically showing a negative correlation between the two.

These hypotheses were tested by statistical analyses of the collected data, focusing on the changes in PTH and FGF23 levels over the study period and the effects of dose adjustments.

3. MATERIALS AND METHODS

3.1 Ethical Considerations and Study Design

The study was a multicenter, randomized, double-blind, prospective, crossover trial conducted from January 2009 to November 2010. The primary objective was to assess the dose-response relationship of paricalcitol on parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels in patients with end-stage renal failure undergoing chronic intermittent hemodialysis. This study was part of the EPIC-CKD study, which stands for "Effects of Paricalcitol on Inflammation and Calcification regulation in Chronic Kidney Disease." The EPIC-CKD study aimed to investigate the effects of Paricalcitol capsules on inflammation, measured by CRP levels, and calcification regulation, measured by fetuin-A levels, in patients with CKD stage 5D (Eudract 2007-006606-16). The data presented in this thesis has not been previously analyzed or published in this form. Ethical approval was obtained from the Ethics Committee of the University of Würzburg, ensuring that all procedures adhered to the ethical guidelines established by the responsible committee for human experimentation, in line with the Helsinki Declaration of 1975, as amended in 2000.

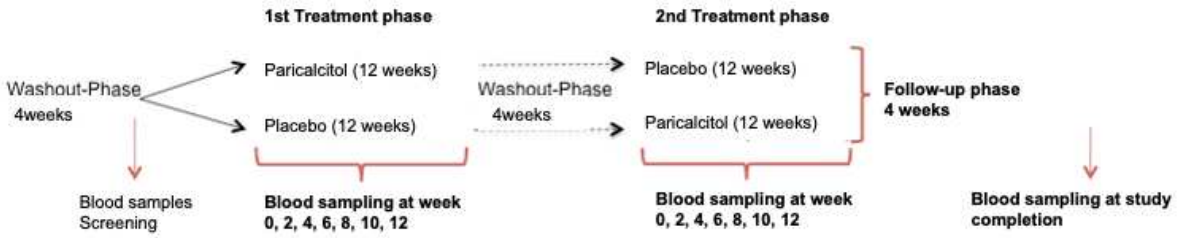

Figure 1. Study Design

3.2 Participants

A total of 44 patients were screened, and 43 were recruited into the study. Inclusion criteria were: age ≥18 years, on dialysis for more than six months but less than five years, no active vitamin D analog treatment within one month of study screening, PTH levels between 200-600 pg/mL, serum calcium <2.55 mmol/L, phosphorus <2.1 mmol/L, and 25 OH-vitamin D >20 ng/mL. Exclusion criteria included acute infections, malignancy, heart failure, and calcimimetic therapy.

3.3 Intervention

Participants were randomized to receive either paricalcitol or placebo for 12 weeks, followed by a 4-week washout period, after which they crossed over to the other treatment arm for another 12 weeks. The initial dose of paricalcitol was 2 μg/day. The dosage was adjusted based on individual patient response, with the average cohort dosage being approximately 1.9 μg/day at week six and 1.5 μg/day at week twelve. Blood samples were collected at baseline and every two weeks during the treatment phases.

3.4 Outcome Measures

The primary outcome measures were the changes in PTH and FGF23 levels from baseline to weeks six and twelve. Secondary outcomes included the effects of dose reduction on these hormone levels.

3.5 Statistical Analysis

Data were analyzed using a mixed model repeated measures (MMRM) approach, including fixed effects for treatment, treatment sequence, period, visit, baseline value, treatment x visit interaction, baseline x visit interaction, and including site and subject as random effects. Least squares means (LSM) were calculated for both PTH and FGF23 levels at various time points. Only patients with FGF23 data on paricalcitol on both Weeks 6 and 12 are considered. Statistical analyses were performed using SAS software (Statistical Analysis System) from SAS Institute Inc., Cary, North Carolina, USA. A P-value of less than 0.05 was considered statistically significant.

3.6 Dosing Information

For the 31 patients with FGF23 and/or PTH measurements at both week six and week twelve during the paricalcitol treatment phase, the dosing information was as follows: at Week 6, 5 patients were on 1 μg/day, 25 patients were on 2 μg/day, and 1 patient was on 3 μg/day, with a mean cohort dosage of 1.871 μg/day. At Week 12, 4 patients were on 0.5 μg/day, 9 patients were on 1 μg/day, 17 patients were on 2 μg/day, and 1 patient was on 3 μg/day, with a mean cohort dosage of 1.548 μg/day (Dosages, week 6 and 12). This comprehensive methodology ensures the reliability and validity of the study's findings on the effects of

paricalcitol in managing secondary hyperparathyroidism in patients with end-stage renal failure.

3.7 Baseline Characteristics

Table 1. The baseline characteristics of the participants

Characteristic	Overall (n=43)	Sequence A (n=22) Paricalcitol - Placebo	Sequence B (n=21) Paricalcitol - Placebo
Age (years)	63.6 (10.4)	62.1 (9.4)	65.1 (11.4)
Female Gender	13 (30.2%)	6 (27.3%)	7 (33.3%)
Weight (kg)	87.9 (15.9)	88.9 (15.6)	86.8 (16.5)
Height (cm)	169.8 (7.9)	170.7 (8.2)	168.8 (7.5)
Body-Mass-Index (kg/m²)	30.5 (5.4)	30.6 (5.5)	30.4 (5.4)
Systolic Blood Pressure (mmHg)	135.1 (16.9)	135.6 (19.3)	134.5 (14.4)
Diastolic Blood Pressure (mmHg)	74.4 (10.7)	74.7 (11.5)	74.0 (10.1)
Heart Rate (beats/min)	70.5 (9.1)	71.4 (9.6)	69.7 (8.6)
Dialysis Duration (h)	4.89 (0.95)	4.84 (0.85)	4.95 (1.06)
Dialysis Frequency per Week	3 (0)	3 (0)	3 (0)
Weekly Dialysis Duration (h)	14.68 (2.85)	14.52 (2.55)	14.84 (3.18)
Dialysis Efficiency (Kt/V[a])	1.40 (0.21)	1.32 (0.19)	1.48 (0.20)
Dialysate Calcium (mmol/l)	1.37 (0.14)	1.38 (0.15)	1.37 (0.13)
Hemoglobin (g/dl)	12.10 (1.07)	12.54 (0.85)	11.63 (1.09)
Serum Phosphate (mmol/l)	1.57 (0.36)	1.60 (0.40)	1.53 (0.33)
Intact PTH (pmol/l[b])	33.84 (13.23)	33.89 (15.24)	33.79 (11.25)
25-OH Vitamin D (ng/ml)	37.14 (15.2)	35.98 (17.94)	38.35 (12.01)
Serum Albumin (g/dl)	4.01 (0.33)	4.08 (0.37)	3.94 (0.28)
Diabetes mellitus	21 (48.8%)	9 (40.9%)	12 (57.1%)
Arterial Hypertension	41 (95.3%)	21 (95.5%)	20 (95.2%)

Characteristic	Overall (n=43)	Sequence A (n=22) Paricalcitol - Placebo	Sequence B (n=21) Paricalcitol - Placebo
Coronary Artery Disease	18 (41.9%)	11 (50%)	7 (33.3%)
Peripheral Artery Disease	11 (25.6%)	8 (36.4%)	3 (14.3%)
TIA[c] or Stroke	6 (14%)	4 (18.2%)	2 (9.5%)
Chronic Inflammatory Conditions	14 (32.6%)	9 (40.9%)	5 (23.8%)
Chronic Glomerulonephritis	8 (18.6%)	7 (31.8%)	1 (4.8%)
Other[d]	7 (16.3%)	3 (13.6%)	4 (19.0%)
Use of ESA	38 (88.4%)	18 (81.8%)	20 (95.2%)
Iron Substitution	33 (76.7%)	18 (81.8%)	15 (71.4%)
Anti-inflammatory Medication	39 (90.7%)	19 (86.4%)	20 (95.2%)
Statins	24 (55.8%)	13 (59.1%)	11 (52.4%)
Acetylsalicylic Acid	33 (76.7%)	16 (72.7%)	17 (81%)
Beta-blockers	31 (72.1%)	15 (68.2%)	16 (76.2%)
Sevelamer	3 (7%)	1 (4.5%)	2 (9.5%)
Immunosuppressive	3 (7%)	1 (4.5%)	2 (9.5%)
Phosphate Binders (total)	42 (97.7%)	21 (95.5%)	21 (100%)
Calcium-containing Phosphate Binders	37 (86%)	18 (81.8%)	19 (90.5%)
Lanthanum Carbonate	12 (27.9%)	6 (27.3%)	6 (28.6%)
Aluminum-containing Phosphate Binders	1 (2.3%)	1 (4.5%)	0 (0%)

Data are presented as mean±standard deviation or as number (%)

[a] K=Urea clearance (ml/min), t= Duration of dialysis (h), V=Urea distribution volume (ml)

[b] To convert PTH to pg/ml, multiply by 9.43

[c] Transient Ischemic Attack

[d] Polyarthritis, Chronic Hepatitis, HIV, Psoriasis, M. Wegener, M. Basedow, Hashimoto Thyroiditis, Ulcus cruris

4. RESULTS

4.1 PTH Response to Paricalcitol

The analysis of PTH levels indicated a significant decrease under Paricalcitol treatment at both Week 6 and Week 12 compared to placebo, with no carry-over effects observed (both $p < 0.001$) (Table 2).

Table 2. PTH (ng/L) results least squares mean (LSM)

Time Point	Paricalcitol (LSM)	Placebo (LSM)	Paricalcitol – Placebo (LSM)	*P*
Week 6	161.55	357.02	-195.47	<0.001
Week 12	152.93	361.43	-208.49	<0.001

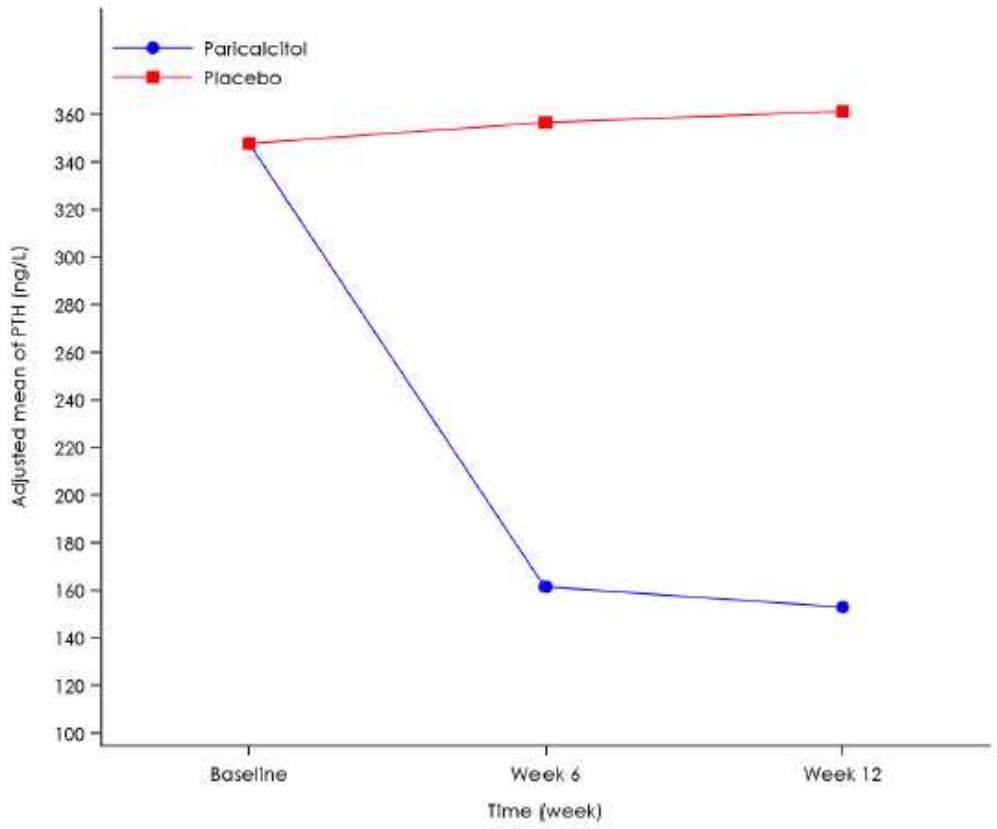

Figure 2. Adjusted means of parathormone (PTH) levels across time

Figure 2 shows the adjusted means of PTH levels across time, highlighting the significant changes associated with dose reduction.

4.2 FGF23 Response to Paricalcitol

The analysis of FGF23 levels showed a significant increase under paricalcitol treatment at Week 6 compared to placebo (p = 0.002) (Table 3). However, by Week 12, the difference between the paricalcitol and placebo groups was no longer significant (p = 0.242), although the values under paricalcitol remained elevated compared to placebo and baseline levels (Table 3). No carry-over effects were observed.

Table 3. FGF23 (log-transformed) and PTH results least squares mean (LSM)

Time Point	Paricalcitol (LSM)	Placebo (LSM)	Paricalcitol – Placebo (LSM)	*P*
FGF23 (kRU/l log-transformed data) n = 36 subjects 121 observations baseline LSM = 5.611				
Week 6	6.497	5.779	0.717	0.002
Week 12	5.957	5.722	0.235	0.242
PTH (ng/L) n = 41 subjects 136 observations baseline LSM = 347.85				
Week 6	161.55	357.02	-195.47	<0.001
Week 12	152.93	361.43	-208.49	<0.001

Analyses are based on a mixed model repeated measures (MMRM) including fixed effects for treatment, treatment sequence, period, visit, baseline value, treatment x visit interaction, baseline x visit interaction and including site and subject as random effects.

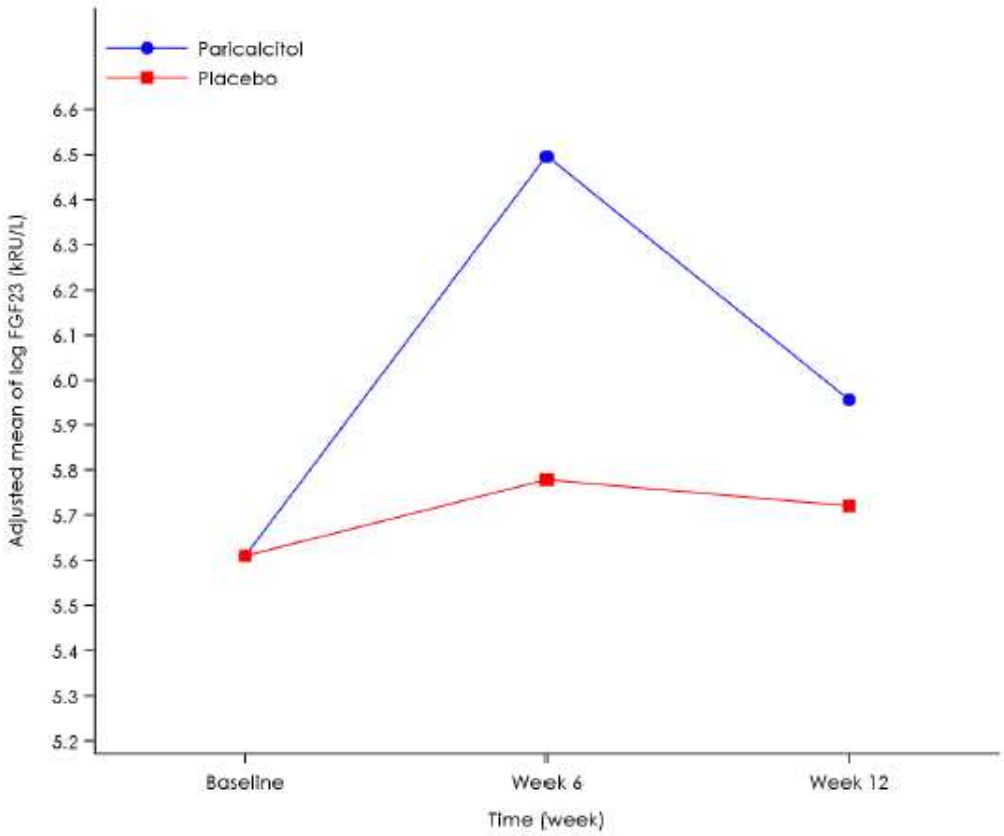

Figure 3. Adjusted means of log-transformed FGF23 across time.

Figure 3 illustrates the adjusted means of log-transformed FGF23 across time, showing the initial significant increase at Week 6 and the subsequent stabilization by Week 12.

4.3 Effect of Paricalcitol Dose Reduction

The impact of dose reduction of paricalcitol after the Week 6 visit was also examined. Patients were divided into two groups: those who had their dose reduced and those who maintained a stable dose. The results showed a decrease in FGF23 levels ($p = 0.014$) and an increase in PTH levels ($p < 0.001$) among patients who reduced their Paricalcitol dose (Table 4).

Table 4. FGF23 (log-transformed) and PTH comparing Week 6 and Week 12 values between patients with paricalcitol dose reduction and patients with stable paricalcitol dose

Time Point	Dose Reduction (n, mean)	Stable Dose (n, mean)	Reduced – Stable Dose (mean)	*P*
FGF23 (kRU/l log-transformed data)				
Week 12 – Week 6	n=10, mean=-1.277	n=21, mean=-0.239	-1.038	0.014
PTH (ng/L)				
Week 12 – Week 6	n=9, mean=78.87	n=20, mean=-57.55	136.43	<0.001

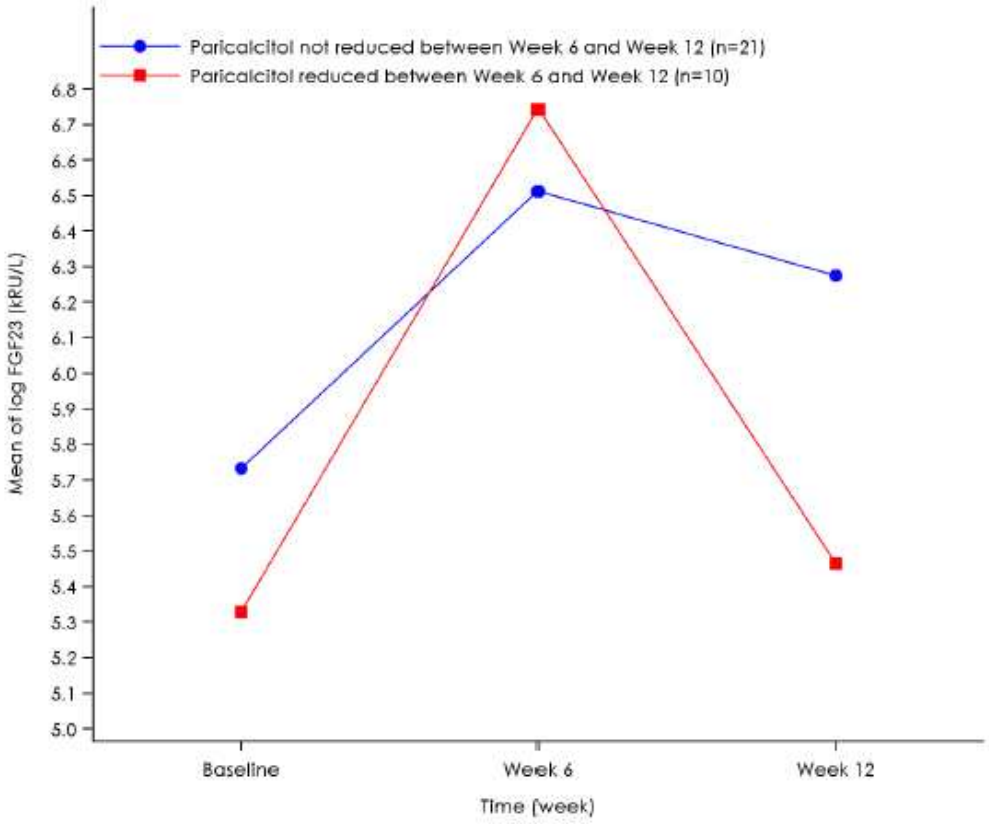

Figure 4. Means of log-transformed FGF23 across time, by paricalcitol dose change

Figure 4 shows the adjusted means of PTH levels across time, highlighting the significant reduction at both Week 6 and Week 12 under paricalcitol treatment.

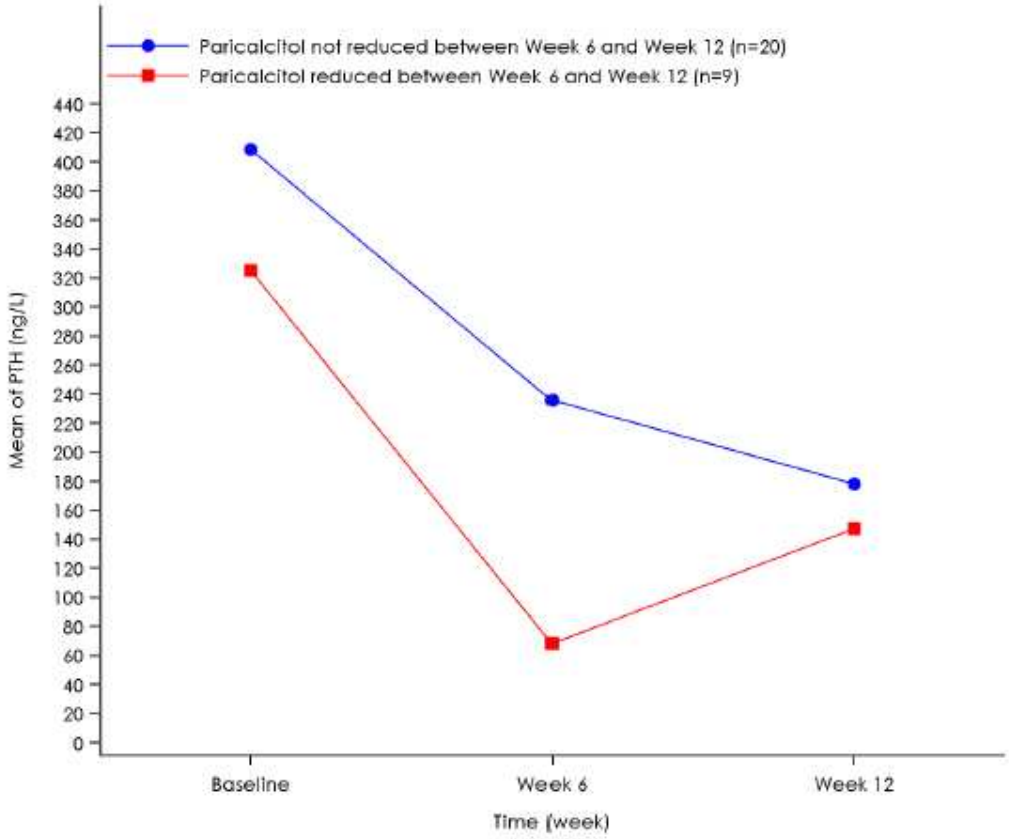

Figure 5. Means of parathormone (PTH) across time, by paricalcitol dose change

Figure 5 demonstrates the means of parathyroid hormone (PTH) levels over time, categorized by whether patients experienced a reduction in their paricalcitol dose between Week 6 and Week 12. Initially, both groups showed a significant decline in PTH levels from baseline to Week 6. However, by Week 12, those patients whose paricalcitol dose was reduced between Week 6 and Week 12 exhibited a slight rebound in PTH levels. Despite this increase, the PTH levels in this group remained markedly lower than at baseline and were still within the target range. In contrast, patients who maintained a stable paricalcitol dose continued to see a decrease in PTH levels, indicating the importance of consistent dosing for sustained suppression of PTH levels.

4.4 Summary of Findings

Paricalcitol highly significantly increased FGF23 levels at Week 6 but not at Week 12, although final FGF23 levels were slightly, non-significantly elevated compared to baseline (Table 3, Figure 3). Paricalcitol significantly decreased PTH levels at both Week 6 and Week 12 (Table 2, Figure 2). Dose reduction of paricalcitol was associated with a decrease in FGF23

levels and an increase in PTH levels, indicating a negative correlation between changes in FGF23 and PTH levels (Table 4, Figure 4). However, PTH levels at 12 weeks still remained well within the target range and were still markedly lower than at baseline (Figure 5). These findings highlight the complex interactions between paricalcitol dosing and the regulation of FGF23 and PTH in patients with end-stage renal failure on chronic intermittent hemodialysis. Further research is needed to optimize dosing strategies and understand the long-term implications of these hormonal changes.

5. DISCUSSION

The present study aimed to examine the dose-response relationship of paricalcitol on parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels in patients with end-stage renal failure undergoing chronic intermittent hemodialysis. The findings provide significant insights into the hormonal regulation by paricalcitol in this patient population.

The study demonstrated that paricalcitol administration significantly reduced PTH levels at both six and twelve weeks compared to placebo. This finding aligns with previous studies indicating that paricalcitol is effective in managing secondary hyperparathyroidism (sHPT) in patients with chronic kidney disease (CKD) (49). The sustained suppression of PTH levels over twelve weeks suggests that paricalcitol maintains its efficacy over the treatment period, even though the dosage was reduced.

Paricalcitol significantly increased FGF23 levels at six weeks, but this effect was not significant at twelve weeks. This transient increase in FGF23 could be attributed to the body's initial response to the active vitamin D analogue. However, it is also possible that the stabilization of FGF23 levels was influenced by the reduction in paricalcitol dosage after six weeks, which might have lessened its stimulatory effect on FGF23 production. The non-significant change in FGF23 at twelve weeks suggests that both an adaptive physiological response and the adjusted dosing of paricalcitol may have played roles in mitigating the initial rise in FGF23 levels.

The study also highlighted the effects of paricalcitol dose reduction after six weeks. Patients who reduced their dose showed a decrease in FGF23 levels and an increase in PTH levels. However, PTH levels remained well within the target range. It is worthy to note that if the paricalcitol dosage had not been reduced, hypoparathyroidism may have resulted. This inverse relationship underscores the importance of maintaining an appropriate dosage of paricalcitol to balance the hormonal levels effectively. The findings of this study have several clinical implications. Paricalcitol is confirmed as an effective treatment for reducing PTH levels in patients with sHPT, which can help mitigate the risks associated with high PTH levels, such as bone disease and cardiovascular complications (7). Given the initial increase in FGF23 levels, it appears prudent to monitor FGF23 in patients receiving paricalcitol, particularly in the early stages of treatment. This monitoring can potentially help identify any adverse cardiovascular risks associated with elevated FGF23. The study suggests that dose adjustments are warranted for maintaining the efficacy of paricalcitol. Reducing the dose after an initial period can help manage FGF23 levels without significantly compromising the suppression of PTH.

The findings of this study align with other research, which also reported the effectiveness of paricalcitol in reducing PTH levels (49). A systematic review and meta-analysis by Takkavatakarn *et al.* (2021) evaluated the effectiveness of various interventions in managing CKD, particularly in relation to FGF23 levels. The study highlighted that non-calcium-based phosphate binders, iron supplements, and calcimimetics were effective in reducing FGF23 levels. While the review did not specifically focus on paricalcitol, it underscored the complexity of managing FGF23 levels in CKD patients, suggesting that a combination of therapies may be necessary to optimize patient outcomes (50). This aligns with the need for careful dose management of paricalcitol, as its effects on FGF23 may vary and require adjunctive treatments for the best therapeutic results. Another review highlighted the cardiovascular risks associated with elevated FGF23 levels in CKD patients, noting that FGF23, along with phosphate, acts as a cardiovascular toxin, promoting hypertension, vascular calcification, and left ventricular hypertrophy (42). This underscores the need for careful management of FGF23 levels in CKD patients receiving paricalcitol.

When interpreting the results of this study, several limitations must be taken into account. The relatively small sample size may impact the generalizability of the findings, and larger studies are needed to validate these results. The study duration of twelve weeks may not capture the long-term effects of paricalcitol on PTH and FGF23 levels, so long-term studies are necessary to understand the chronic impacts of paricalcitol treatment. Although the study was multicenter, the majority of data was derived from a single center, which may introduce center-specific biases. However, the data were collected in a randomized, double blinded and cross-over mode and were, thus, of high quality. Future research should focus on investigating the long-term effects of paricalcitol on PTH and FGF23 levels to provide a comprehensive understanding of its impact on hormonal regulation. It should also explore the relationship between FGF23 levels and cardiovascular outcomes in patients receiving paricalcitol to determine the clinical significance of the observed hormonal changes. Additionally, developing optimal dosing strategies for paricalcitol that balance the suppression of PTH with the management of FGF23 levels to minimize potential risks is crucial.

In conclusion, paricalcitol effectively reduces PTH levels in patients with end-stage renal failure on chronic intermittent hemodialysis. While it initially increases FGF23 levels, this effect is not sustained over twelve weeks. Dose adjustments are essential to maintain the therapeutic efficacy of paricalcitol and manage the hormonal balance. Further research is needed to explore the long-term implications and optimize treatment strategies for patients with CKD.

6. CONLCUSIONS

This study aimed to explore the dose-response relationship of paricalcitol on parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels in patients with end-stage renal failure undergoing chronic intermittent hemodialysis. The findings provide significant insights into the hormonal regulation by paricalcitol in this patient population.

1. **PTH Reduction:** Paricalcitol administration resulted in a significant reduction in PTH levels at both six and twelve weeks compared to placebo. This indicates that paricalcitol is effective in managing secondary hyperparathyroidism in patients with end-stage renal failure requiring lowering of the dosage in the latter phase of the study to maintain PTH in the recommended target range.
2. **FGF23 Increase:** Paricalcitol significantly increased FGF23 levels at six weeks compared to baseline and placebo. However, this increase was not significant at twelve weeks, suggesting a transient effect of paricalcitol on FGF23 levels.
3. **Impact of Dose Reduction:** Patients who reduced their paricalcitol dose after six weeks experienced a decrease in FGF23 levels and an increase in PTH levels, indicating a negative correlation between the two hormones. This highlights the importance of dose management in maintaining the therapeutic effects of paricalcitol.
4. **Clinical Implications:** The results underscore the need for careful monitoring and adjustment of paricalcitol dosage to optimize its benefits on PTH and FGF23 levels, potentially improving clinical outcomes in patients with end-stage renal failure.

In conclusion, paricalcitol is effective in reducing PTH levels and has a transient, albeit dose dependent effect on increasing FGF23 levels. Dose adjustments are crucial for maintaining its efficacy and managing the hormonal balance in patients undergoing chronic intermittent hemodialysis. Further research is needed to explore the long-term implications of these findings and optimize treatment strategies. Based on the findings from this study and the review of the literature, the dosing of paricalcitol for patients with end-stage renal failure on chronic intermittent hemodialysis should consider the following:

1. **Initial Dosing:** It might be more prudent to start with a lower dose of 1.5 µg/day (=10.5 µg/week) of paricalcitol, given the potential for high FGF23 levels with higher doses.
2. **Monitoring and Adjustments:** Monitor PTH and FGF23 levels initially every two weeks. If PTH levels are significantly reduced but FGF23 levels rise markedly, consider slightly reducing the dose.

3. **Dose Adjustment Strategy:** If the initial dosing at 1.5 μg/day (=10.5 μg/week) is well-tolerated and effective in reducing PTH without a significant rise in FGF23, this dose can be maintained. Otherwise, incremental adjustments can be made based on the patient's hormonal responses.

In summary, starting with a lower dose, such as 1.5 μg/day (=10.5 μg/week), may help mitigate the initial spike in FGF23 while still effectively managing PTH levels. This approach allows for fine-tuning the dose based on individual patient responses, aiming to balance the benefits and potential risks.

7. REFERENCES

1. Wilson S, Mone P, Jankauskas SS, Gambardella J, Santulli G. Chronic kidney disease: Definition, updated epidemiology, staging, and mechanisms of increased cardiovascular risk. J Clin Hypertens (Greenwich). 2021;4:831-834
2. Gaitonde DY, Cook DL, Rivera IM. Chronic Kidney Disease: Detection and Evaluation. Am Fam Physician. 2017;12:776-783.
3. Vaidya SR, Aeddula NR. Chronic Kidney Disease [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [Updated 2024 Jul 31; cited 2024 July 20]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535404/
4. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. Kidney Int Suppl. 2022;1:7-11.
5. Center for Disease Control C [Internet] . Atlanta GA: Chronic Kidney Disease in the United States; 2023 [updated 2024 June cited 2024 Aug 1]. Available from: https://www.cdc.gov/kidneydisease/publications-resources/CKD-national-facts.html
6. Saliba W, El-Haddad B. Secondary hyperparathyroidism: pathophysiology and treatment. J Am Board Fam Med. 2009;5:574-81.
7. Muppidi V, Meegada SR, Rehman A. Secondary Hyperparathyroidism. [Internet]. Treasure Island (FL): StatPearls Publishing; [Updated 2023 Aug 28; cited June 30]. . Available from: https://www.ncbi.nlm.nih.gov/books/NBK557822/
8. AMBOSS [Internet]. New York USA: AMBOSS; 2023. chronic kidney disease; 2023 [cited 2024 Aug 20]. Available from: https://next.amboss.com/us/article/lg0vv2?q=chronic+kidney+disease
9. Kanwar YS, Sun L, Xie P, Liu FY, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. Annual Review of Pathology: Mechanisms of Disease. 2011;6:395-423.
10. Bidani AK, Griffin KA. Pathophysiology of hypertensive renal damage: implications for therapy. Hypertension. 2004;5:595-601.
11. Bonsib SM. Non-neoplastic Diseases of the Kidney. Genitourinary Pathology. 2021;4: 281-286.
12. Fujimoto T. Pathology of malignant nephrosclerosis with special reference to the difference between histologic manifestations of pure and exacerbated forms. Tohoku J Exp Med. 1978;2:135-53.
13. Kazi AM, Hashmi MF. Glomerulonephritis. 2023 Jun 26.[Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 July 20]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560644/

14. Usherwood T, Lee V. Advances in chronic kidney disease pathophysiology and management. Aust J Gen Pract. 2021;4:188-192.
15. Zemaitis MR, Foris LA, Katta S, et al. Uremia. [Internet] Treasure Island (FL). StatPearls Publishing; 2024 Jan; cited July 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441859/
16. Courbebaisse M, Lanske B. Biology of fibroblast growth factor 23: From physiology to pathology. Cold Spring Harb Perspect Med. 2018;5: a031260.
17. Pavlovic D, Katicic D, Gulin T, Josipovic J. Vitamin D in the Patients with Chronic Kidney Disease: When, to Whom and in Which Form. Materia Socio Medica. 2015;2:122-124.
18. Aleissa M, Alghofaili I, Alotaibe H, Yaslam M, Almujil M, Arnous M, et al. Incidence and risk factors associated with hypoglycemia among patients with chronic kidney disease: A systematic review. Vol. 27, Journal of Family and Community Medicine. Wolters Kluwer Medknow Publications. 2020;3:157-62.
19. Chen TK, Knicely DH, Grams ME. Chronic Kidney Disease Diagnosis and Management: A Review. Vol. 322, JAMA - Journal of the American Medical Association. American Medical Association. 2019;13:1294-304.
20. Huang MJ, Wei RB, Wang Y, Su TY, Di P, Li QP, et al. Blood coagulation system in patients with chronic kidney disease: A prospective observational study. BMJ Open. 2017;5:e014294.
21. Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update. American Journal of Kidney Diseases. 2020;3:1-107.
22. Kim SM, Jung JY. Nutritional management in patients with chronic kidney disease. Vol. 35, Korean Journal of Internal Medicine. Korean Association of Internal Medicine. 2020;6:279-1290.
23. Stevens PE, Levin A; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Ann Intern Med. 2013;11:825-30.
24. Kidney Disease: Improving Global Outcomes (KDIGO) Blood Pressure Work Group. KDIGO 2021 Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. Kidney Int. 2021;3:S1-S87.

25. Lee T, Kim WK, Kim AJ, Ro H, Chang JH, Lee HH, Chung W, Jung JY. Low-Osmolar vs. Iso-Osmolar Contrast Media on the Risk of Contrast-Induced Acute Kidney Injury: A Propensity Score Matched Study. Front Med (Lausanne). 2022;9:862023.
26. International journal of nephrology. Supplement to Kidney International. 35th ed. Belgium: Elsevier: 2021. 37-55.
27. Mikolasevic I, Žutelija M, Mavrinac V, Orlic L. Dyslipidemia in patients with chronic kidney disease: Etiology and management. Vol. 10, International Journal of Nephrology and Renovascular Disease. Dove Medical Press Ltd. 2017;10: 35-45.
28. Development Work Group Members. KDIGO Clinical Practice Guideline for Lipid Management in CKD: summary of recommendation statements and clinical approach to the patient. Kidney Int. 2014;6:1303-9.
29. de Boer IH, Caramori ML, Chan JCN, Heerspink HJL, Hurst C, Khunti K, et al. Executive summary of the 2020 KDIGO Diabetes Management in CKD Guideline: evidence-based advances in monitoring and treatment. Kidney Int. 2020;94:839-48.
30. Natale P, Palmer SC, Ruospo M, Saglimbene VM, Rabindranath KS, Strippoli GFM. Psychosocial interventions for preventing and treating depression in dialysis patients. Vol. 2019, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd. 2019;12: CD004542.
31. Shroff R, Wan M, Nagler E V., Bakkaloğlu S, Cozzolino M, Bacchetta J, et al. Clinical practice recommendations for treatment with active Vitamin D analogues in children with chronic kidney disease Stages 2-5 and on dialysis. Nephrology Dialysis Transplantation. 2017;7:1114-27.
32. Amphansap T, Therdyothin A, Stitkitti N, Nitiwarangkul L, Phiphobmongkol V. Efficacy of plain cholecalciferol versus ergocalciferol in raising serum vitamin D level in Thai female healthcare workers. Osteoporos Sarcopenia. 2022;4:145-51.
33. Jiménez-Cortegana C, Sánchez-Martínez PM, Palazón-Carrión N, Nogales-Fernández E, Henao-Carrasco F, García-Sancho AM, et al. Lower survival and increased circulating suppressor cells in patients with relapsed/refractory diffuse large B-cell lymphoma with deficit of vitamin D levels using R-GDP plus lenalidomide (R2-GDP): Results from the R2-GDP-gotel trial. Cancers (Basel). 2021:13;18.
34. Lips P. Vitamin D physiology. Vol. 92, Progress in Biophysics and Molecular Biology. 2006;1:4-8.
35. AMBOSS [Internet]. New York USA: AMBOSS; Vitamins; 2022 [cited 2024 Aug 15]. Available from: https://next.amboss.com/us/article/Ao0ReS?q=vitamins

36. Gonzalez-Parra E, Rojas-Rivera J, Tuñón J, Praga M, Ortiz A, Egido J. Vitamin D receptor activation and cardiovascular disease. Nephrology Dialysis Transplantation. 2012:4;17-21.
37. Eisman JA, Bouillon R. Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. Bonekey Rep.2014;3:499.
38. Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R. The new england journal of medicine.2003;349:446-456.
39. Khan M, Jose A, Sharma S. Physiology, Parathyroid Hormone. [Internet]. Treasure Island (FL). StatPearls Publishing; 2024 [Updated 2022 Oct 29:cited 2024 June 20]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499940/
40. AMBOSS [Internet]. New York USA: AMBOSS; Hypocalcemia; 2023 [cited 2024 Aug 19]. Available from: https://next.amboss.com/us/article/Hg0K92?q=hypocalcemia
41. Naveh-Many T, Volovelsky O. Parathyroid cell proliferation in secondary hyperparathyroidism of chronic kidney disease. Vol. 21. International Journal of Molecular Sciences 2020:11:1-17.
42. Vogt I, Haffner D, Leifheit-Nestler M. FGF23 and Phosphate-Cardiovascular Toxins in CKD. Toxins (Basel). 2019;11:647.
43. Mihai R, Farndon JR. Parathyroid disease and calcium metabolism. Br J Anaesth. 2000;1:29-43.
44. Rausch S, Föller M. The regulation of FGF23 under physiological and pathophysiological conditions. Pflugers Arch. 2022;3:281-292.
45. Hidaka N, Inoue K, Kato H, Hoshino Y, Koga M, Kinoshita Y, et al. FGF-23, Left Ventricular Hypertrophy, and Mortality in Patients With CKD: A Revisit With Mediation Analysis. JACC: Advances. 2024;1:100747.
46. Musgrove J, Wolf M. Regulation and Effects of FGF23 in Chronic Kidney Disease. Annu Rev Physiol. 2020;82:365-390
47. Liu T, Wen H, Li H, Xu H, Xiao N, Liu R, et al. Oleic Acid Attenuates Ang II (Angiotensin II)-Induced Cardiac Remodeling by Inhibiting FGF23 (Fibroblast Growth Factor 23) Expression in Mice. Hypertension.2020;3:680-92.
48. Olgaard K, Lewin E, Silver J. Calcimimetics, vitamin D and ADVANCE in the management of CKD-MBD. Nephrology Dialysis Transplantation. 2011;4:1117-9.
49. Han T, Rong G, Quan D, Shu Y, Liang Z, She N, Liu M, Yang B, Cheng G, Lv Y, Stern L. Meta-analysis: the efficacy and safety of paricalcitol for the treatment of secondary

hyperparathyroidism and proteinuria in chronic kidney disease. Biomed Res Int. 2013;2013:320560.

50. Takkavatakarn K, Wuttiputhanun T, Phannajit J, Praditpornsilpa K, Eiam-Ong S, Susantitaphong P. Effectiveness of fibroblast growth factor 23 lowering modalities in chronic kidney disease: a systematic review and meta-analysis. Int Urol Nephrol. 2022;2:309-21.

8. SUMMARY

Objectives: The aim of this study was to determine the dose response of PTH and FGF23 to Paricalcitol in patients with end stage renal failure on chronic intermittent haemodialysis. This study was part of the EPIC-CKD study, which focused on the effects of Paricalcitol capsules on inflammation (measured by CRP levels) and calcification regulation (measured by fetuin-A levels) in CKD stage 5D patients.

Patients and methods: This multicenter, randomized, double-blind, prospective, crossover EPIC-CKD study enrolled a total of 43 CKD stage 5D patients. The inclusion criteria were: age ≥18 years, on dialysis for more than six months but less than five years, no active vitamin D analog treatment within one month of study screening, PTH levels between 200-600 pg/mL, serum calcium <2.55 mmol/L, phosphorus ≤2.1 mmol/L, and 25 OH-vitamin D >20 ng/mL. Exclusion criteria included acute infections, malignancy, heart failure, and calcimimetic therapy. A total of 31 complete patient data sets were available for analysis.

Results: Patients started with an initial dose of paricalcitol at 2 µg/day, adjusted to 1.9 µg/day at week six and 1.5 µg/day at week twelve. FGF23 levels showed a significant increase at six weeks ($p = 0.002$), while the final FGF23 levels at twelve weeks were not significantly different from the baseline ($p = 0.242$). PTH levels remained significantly suppressed at both six and twelve weeks (both $p < 0.001$). Subgroup analysis revealed that dose reduction in paricalcitol led to a statistically significant increase in PTH, paralleling a decrease in FGF23, although the absolute PTH rebound from an over-suppressed range was moderate compared to the absolute decrease in FGF23.

Conclusions: This study provides significant insights into the hormonal regulation by paricalcitol in patients with end-stage renal failure. Paricalcitol administration resulted in a significant reduction in PTH levels at both six and twelve weeks compared to placebo, demonstrating its effectiveness in managing secondary hyperparathyroidism. The study also observed a significant increase in FGF23 levels at six weeks, which was not sustained at twelve weeks, indicating a transient effect of paricalcitol on FGF23. Moreover, dose reduction after six weeks led to a significant decrease in FGF23 levels and a moderate increase in PTH levels, illustrating the importance of dose management to maintain therapeutic effects. These findings underscore the necessity for careful monitoring and adjustment of paricalcitol dosage to optimize its benefits on PTH and FGF23 levels, potentially improving clinical outcomes in

patients with end-stage renal failure. Based on these findings, it is advisable to start with a lower dose of 1.5 µg/day (=10.5 µg/week) of paricalcitol to mitigate the initial spike in FGF23 while effectively managing PTH levels. Monitoring PTH and FGF23 levels every two weeks is crucial, and maintaining or slightly reducing the dose should be considered if FGF23 levels rise. This approach allows for fine-tuning the dose based on individual patient responses, aiming to balance the benefits and potential risks.

9. CROATIAN SUMMARY

Naslov: Odgovor PTH i FGF23 na Parikalcitol kod pacijenata s terminalnim zatajenjem bubrega na kroničnoj intermitentnoj hemodijalizi

Ciljevi: Cilj ovog istraživanja bio je utvrditi dozni odgovor paratiroidnog hormona (PTH) i fibroblastnog čimbenika rasta 23 (FGF23) na Parikalcitol kod pacijenata s terminalnim stadijem zatajenja bubrega na kroničnoj intermitentnoj hemodijalizi. Ovo istraživanje je dio EPIC-CKD studije, koja se fokusirala na učinke Parikalcitol kapsula na upalu (mjereno razinama CRP-a) i regulaciju kalcifikacije (mjereno razinama fetuin-A) kod pacijenata u stadiju 5D kronične bubrežne bolesti (CKD).

Pacijenti i metode: Ova multicentrična, randomizirana, dvostruko slijepa, prospektivna, crossover EPIC-CKD studija obuhvatila je ukupno 43 pacijenta u stadiju 5D kronične bubrežne bolesti. Uključni kriteriji bili su: dob ≥18 godina, trajanje dijalize više od šest mjeseci ali manje od pet godina, bez prethodne aktivne terapije vitaminom D unutar mjesec dana od pregleda, razine PTH između 200-600 pg/mL, serumskog kalcija <2,55 mmol/L, fosfora ≤2,1 mmol/L i 25 OH-vitamina D >20 ng/mL. Isključni kriteriji uključivali su akutne infekcije, malignitet, zatajenje srca i terapiju kalcimimeticima. Ukupno su bila dostupna 31 kompletna podatkovna seta za analizu.

Rezultati: Pacijenti su započeli s početnom dozom parikalcitola od 2 µg/dan, smanjenom na 1,9 µg/dan nakon šest tjedana i na 1,5 µg/dan nakon dvanaest tjedana. Razine FGF23 pokazale su značajan porast nakon šest tjedana ($p = 0,002$), dok konačne razine FGF23 nakon dvanaest tjedana nisu bile značajno različite od početnih ($p = 0,242$). Razine PTH ostale su značajno potisnute i nakon šest i nakon dvanaest tjedana (oboje $p < 0,001$). Subgrupna analiza pokazala je da je smanjenje doze parikalcitola dovelo do statistički značajnog povećanja PTH uz paralelno smanjenje FGF23. Međutim, apsolutni povrat PTH iz prekomjerno potisnutog raspona bio je umjeren u usporedbi s apsolutnim smanjenjem FGF23.

Zaključci: Ova studija pruža značajne uvide u hormonalnu regulaciju putem parikalcitola kod pacijenata s terminalnim stadijem zatajenja bubrega. Primjena parikalcitola rezultirala je značajnim smanjenjem razina PTH u šestom i dvanaestom tjednu u usporedbi s placebom, čime se potvrđuje njegova učinkovitost u upravljanju sekundarnim hiperparatireoidizmom. Studija je također zabilježila značajan porast razina FGF23 u šestom tjednu, koji se nije održao do dvanaestog tjedna, što ukazuje na prolazan učinak parikalcitola na FGF23. Nadalje, smanjenje doze nakon šest tjedana dovelo je do značajnog smanjenja razina FGF23 i umjerenog povećanja

razina PTH, što ilustrira važnost upravljanja dozom za održavanje terapijskih učinaka. Ovi nalazi naglašavaju potrebu za pažljivim praćenjem i prilagodbom doze parikalcitola kako bi se optimizirale njegove koristi na razine PTH i FGF23, potencijalno poboljšavajući kliničke ishode kod pacijenata s terminalnim zatajenjem bubrega. Na temelju ovih nalaza, preporučuje se započeti s nižom dozom od 1,5 μg/dan parikalcitola kako bi se smanjio početni porast FGF23, dok se učinkovito upravlja razinama PTH. Ključno je pratiti razine PTH i FGF23 svaka dva tjedna, a održavanje ili blago smanjenje doze treba razmotriti ako razine FGF23 porastu. Ovaj pristup omogućava fino podešavanje doze na temelju odgovora pojedinih pacijenata, s ciljem balansiranja koristi i potencijalnih rizika.