INCREASED SERUM PHOSPHATE CONCENTRATION WITHIN THE NORMAL REFERENCE LEVELS IS ASSOCIATED WITH ALL-CAUSE MORTALITY IN NON-DIALYSIS CKD PATIENTS: A FIVE-YEAR PROSPECTIVE COHORT STUDY

Ana Cerqueira Janete Quelhas-Santos Núria Paulo Claúdia Camila Dias Manuel Pestana

PII: S0211-6995(25)00062-1

DOI: https://doi.org/doi:10.1016/j.nefro.2025.501352

Reference: NEFRO 501352

To appear in: *NEFROLOGÍA*

Received Date: 18 December 2024

Accepted Date: 12 May 2025

Please cite this article as: Cerqueira A, Quelhas-Santos J, Paulo N, Dias CC, Pestana M, INCREASED SERUM PHOSPHATE CONCENTRATION WITHIN THE NORMAL REFERENCE LEVELS IS ASSOCIATED WITH ALL-CAUSE MORTALITY IN NON-DIALYSIS CKD PATIENTS: A FIVE-YEAR PROSPECTIVE COHORT STUDY (2025), doi: https://doi.org/10.1016/j.nefro.2025.501352

INCREASED SERUM PHOSPHATE CONCENTRATION WITHIN THE NORMAL REFERENCE LEVELS IS ASSOCIATED WITH ALL-CAUSE MORTALITY IN NON-DIALYSIS CKD PATIENTS: A FIVE-YEAR PROSPECTIVE COHORT STUDY

AUTHORS: Ana Cerqueira[1,2,3], Janete Quelhas-Santos[2,3], Núria Paulo[1], Claúdia Camila Dias[2,3], Manuel Pestana [1,2,3]

Affiliations

[1] Unidade Local de Saúde São João, Porto, Portugal

[2] Faculdade de Medicina da Universidade do Porto, Porto, Portugal

[3] RISE – Rede de Investigação em Saúde, Porto, Portugal

Corresponding author:

Ana Cerqueira.

Telephone number: +351 914807726.

e-mail address: ana.cerqueira@ulssjoao.min-saude.pt

Abstract:

Introduction and objectives: Cardiovascular (CV) morbidity and mortality are markedly increased in non-dialysis patients with chronic kidney disease (CKD). Thus, the precise management of CV risk factors involved in CKD is crucial to improving outcomes. Serum phosphate (Pi) and FGF-23 levels have been linked with a higher risk of CV events in CKD. However, the exact thresholds of Pi and FGF-23, at which the risk of adverse events increases remain unknown.

Materials and methods: We evaluated the expression of intact FGF-23 (iFGF-23) and Pi in a non-dialysis CKD patient population (n=82; 42M:40F; median age 61 years) and investigated their association with CV and renal outcomes, in a five-year follow-up period.

Results: At baseline, the median estimated glomerular filtration rate (eGFR), iFGF-23, and Pi were 45 ml/min/1.73m^2 (IQ 26.6-73.1), 69.9 µg/ml (IQ 33-117) and 3.4 mg/dL (IQ 3.3-3.9), respectively. Univariate

analysis showed a strong association of both iFGF-23 and Pi with age, Charlson Comorbidity Index, hypertension, and diabetes. In addition, iFGF-23 and Pi were both associated with the composite outcome (major CV and cerebrovascular events – MACCEs, hospitalizations, and all-cause mortality) during follow-up. Moreover, Pi was independently associated with all-cause mortality during follow-up. The segmentation of the population in terciles, according to Pi (<3mg/dL; 3-3.6 mg/dL; ≥3.7 mg/dL) within reference serum levels, showed a distribution of the fatality of 0%, 20% and 80% (p =0.034), respectively.

Conclusions: Our results reinforce the association of both iFGF-23 and Pi with composite CV outcomes in non-dialysis CKD patients and further suggest that Pi, within current reference levels, may behave as an independent risk factor for mortality in this population. It is suggested that reassessing Pi reference levels for early therapeutic intervention in this population may be justified

Keywords: Chronic kidney disease; Cardiovascular risk; Phosphate; FGF-23

1. Introduction

Chronic kidney disease (CKD) is associated with significantly elevated mortality rates, predominantly driven by cardiovascular (CV) complications, which remain the principal cause of death among these patients (1) (2). Therefore, the early identification of reliable biomarkers capable of predicting adverse outcomes is paramount for improving risk stratification and implementing effective therapeutic strategies to halt disease progression and reduce mortality.

Disruptions in mineral metabolism are a hallmark of CKD, often emerging at its earliest stages (3) (4). Fibroblast growth factor 23 (FGF-23) has garnered considerable attention among the key players. The FGF-23 levels rise before the onset of overt hyperphosphatemia, acting as an early compensatory mechanism to counter phosphate (Pi) retention. Through its interaction with Klotho-FGF receptor complexes in the kidneys, FGF-23 promotes urinary Pi excretion and suppresses the synthesis of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$)(5). Activating Klotho and increasing fractional excretion of phosphate (FEp), serves as a compensatory mechanism to maintain normal serum Pi levels to match dietary intake (6). This response precedes elevations in serum Pi and parathyroid hormone (PTH)(7). Experimental and clinical investigations have implicated FGF-23 in left ventricular hypertrophy (LVH), myocardial fibrosis, soft tissue calcifications, and heart failure, independent of traditional CV risk factors (8),(9). Persistent hyperphosphatemia, secondary hyperparathyroidism, and vitamin D deficiency have been implicated as risk factors for both all-cause and CV mortality disease (10),(11),(12). Nevertheless, the causality of these associations remains a subject of ongoing debate. Although elevated FGF-23 and Pi levels are consistently linked to higher mortality risk in CKD, the precise thresholds at which these factors transition from compensatory to maladaptive remain undefined (13). Recent research suggests that the pathogenic potential of FGF-23 may depend on the co-occurrence of hyperphosphatemia and decreased Klotho expression, a combination frequently observed in advanced CKD (13). Importantly, the early rise in FGF-23 levels—even among normophosphatemic patients—may reflect an underlying inability to effectively regulate Pi homeostasis. This "maladaptive" elevation of FGF-23 could serve as a critical signal, identifying individuals with early-stage CKD who are at higher risk of adverse CV outcomes and who may benefit from intensified Pi management strategies. In addition, prior studies have

highlighted that even Pi concentrations within the upper normal reference range are associated with adverse outcomes, suggesting that subtle disturbances in Pi metabolism may be clinically relevant (14).

However, associations are not causality. Although hyperphosphatemia and FGF-23 are associated with increased mortality in CKD (15),(16) it is not clear whether compensatory increases in FGF-23 secretion induce or protect against the increased CV morbidity and mortality observed in CKD. Furthermore, the precise reference values for FGF-23 and Pi, beyond which adverse effects start to be manifested, remain unclear, and the comprehensive impact of each factor involved is not fully understood. In this study, we aimed to examine the relationship of FGF-23 and serum Pi with other biomarkers of kidney disease and CV risk in a non-dialysis CKD population and sought to explore their role as biomarkers of adverse outcomes and mortality during a five-year follow-up period.

2. Methods

2.1. Study population

Eighty-two non-dialysis CKD patients were recruited from the outpatient clinic of the Nephrology Dept. of Unidade Local de Saúde (ULS) São João, EPE, Porto, Portugal. Patients with acute kidney injury, ongoing immunosuppression, recent hospital admission (<2 weeks), recent infections (<1 week), acute heart failure (diagnosed according to appropriate Framingham criteria), and known psychiatric disturbances were excluded from the study. The etiology of CKD was registered, and patients were distributed according to KDIGO CKD categories, using estimated glomerular filtration rate (eGFR) by the CKD-EPI formula (17). Patients with eGFR ≥60 mL/min/1.73 m² were included if they exhibited persistent albuminuria (urinary albumin-to-creatinine ratio >30 mg/g), reflecting evidence of kidney damage despite preserved eGFR (17).

2.2. Cross-Sectional Study

Anthropometric measurements, resting systolic and diastolic blood pressure, and a validated Charlson Comorbidity Index (CCI) were assessed in all patients. Blood and urine samples were collected from all participants. Renal function, proteinuria, serum Pi, 25-hydroxyvitamin D [25(OH)D], parathormone (PTH), as well as other relevant biomarkers were evaluated using standard laboratory methods. Intact FGF-23 (iFGF-23) levels were assessed by a two-site second-generation ELISA kit (*Immutopics.* San Clemente, CA, USA).

2.3. Prospective Study

All recruited CKD patients were prospectively followed up for a median of 58 (IQ 30-69) months, to evaluate hard renal and CV outcomes including progression of CKD and end-stage renal disease (ESRD), hospitalizations, major adverse CV and cerebral events (MACCEs), and all-cause mortality. The MACCEs included acute coronary syndrome (ACS), acute heart failure, and stroke.

Cardiovascular death was defined as mortality due to a heart-related cause (death attributable to ACS, heart failure, arrhythmia, or sudden death) or to a cerebrovascular event. Hospitalizations included non-programmed, more than 24-hour hospital admissions for medical reasons. Admissions for trauma, surgery, or other scheduled procedures were not considered. A composite CV outcome was established including

MACCEs, hospitalizations, and mortality. Renal outcomes included CKD progression, defined as serum creatinine doubling or a >50% decrease in eGFR according to CKD-EPI formula or renal replacement therapy initiation (ESRD) after enrolment.

2.4. Ethics approval

This study was conducted in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments. The research protocol was reviewed and approved by the Institutional Review Board, including the local Health Ethics Commission and Data Protection Officer of ULSSJ, Porto, Portugal (CES 251.14). Informed consent was obtained from all individual participants included in the study. Respect for privacy rights was always maintained, and all data were anonymised prior to analysis to ensure confidentiality.

2.5. Statistical analysis

Continuous variables were described as minimum, percentile 25, median, percentile 75, and maximum deviations, and categorical variables were presented as absolute (n) and relative frequencies (%). Differences in continuous variables were assessed by the Mann-Whitney *U* test, while chi-square tests were used to analyse differences in categorical variables. A correlation analysis was performed using Spearman correlation coefficients. Logistic regression models were used to visualize the relationships of both iFGF-23 and Pi, with CKD progression, hospitalizations, MACCEs, and all-cause mortality. All reported *p*-values were two-sided, and the significance level was set at 5%. All analyses were conducted using SPSS software (Version 26.0 for Windows, SPSS, Chicago, IL, USA).

3. Results

3.1. Cohort characterization

As shown in Table 1, a total of 82 non-dialysis CKD patients (42M:40F) were enrolled in the study: 29 patients were included in stages 1-2, 25 patients in stages 3a-3b, and 28 patients in stages 4-5. The median age was 61 (IQ 46-69) years. The CKD patients in stages 1-2 were younger than patients in CKD stages 4-5 ($p<0.001$). Proteinuria was observed across all CKD stages, with a trend toward increasing levels as renal function declined ($p=0.004$). The CCI showed a significant increase across the three groups ($p<0.001$). Diabetes and hypertension were both more prevalent in patients with CKD stages 4-5 than in patients with CKD stages 1-2 ($p=0.009$ and $p=0.003$, respectively). No significant differences were observed among the three groups concerning gender, body mass index and baseline CV disease. In addition, left ventricular mass (LVM) and left ventricular ejection fraction (LVEF) did not differ among the three groups at baseline (Table 1).

In this study, parallel with the decrease in eGFR ($p<0.001$), median iFGF-23 levels ($p<0.001$), Pi levels ($p<0.001$), fractional excretion of phosphate (FEp) ($p=0.010$), PTH ($p<0.001$) and BNP ($p=0.030$) progressively increased in the three groups (Table 1). Serum calcium levels were significantly decreased in CKD stages 4-5 in comparison with CKD stages 3 ($p=0.010$), however, no differences were found regarding 25(OH)D) levels, iFGF-23 / 25(OH)D ratio and magnesium levels (Table 1).

No specific therapeutic interventions were implemented in this patient cohort; however, a significant difference in prescription rates was observed exclusively for vitamin D analogues. These agents were more frequently prescribed to patients with CKD stages 4-5.

3.1.1. Cross-Sectional study: Associations of iFGF-23 and Pi with CV-related clinical parameters at baseline.

As shown in Table 2, CV risk factors, including hypertension and diabetes, were significantly associated with iFGF-23 ($p=0.005$ and $p<0.001$, respectively) and Pi ($p=0.027$ and $p=0.009$, respectively) levels at baseline. In addition, iFGF-23 levels were also associated with the presence of CV disease at baseline

(*p*=0.02). (Table 2). Both iFGF-23 and Pi were negatively correlated with eGFR (r=-0.789, p<0.001; r=-0.621, p<0.0019) (Table 3). When we examined the associations of iFGF-23 and Pi, with renal and CV biomarkers we found that iFGF-23 and Pi were both positively correlated with age (r=0.460, p<0.001; r=0.292, p=0.008), CCI (r=0.595, p<0.001; r=0.417, p<0.001) (Table 2); PTH (r=0.595, p<0.001; r=0.506, p<0.001) and BNP (r=0.312, p=0.042; r=0.313, p=0.041) (Table 3).

3.1.2. Prospective Study: Phosphate, within reference levels, is independently associated with all-cause mortality in a five-year follow-up.

The non-dialysis CKD population was prospectively followed up for a median of 58 months (IQ 30-69). During this period, four patients suffered a MACCE (two patients an acute myocardial infarction and two patients a cerebrovascular event); 19 patients experienced renal function deterioration, of which 10 started dialysis; 19 patients were hospitalised for medical reasons, and five patients died, two of them by MACCE. Twenty-three patients reached the combined cardiovascular outcome (MACCEs, hospitalization, and all-cause mortality) (Table 4).

To assess the prognostic value of these mineral metabolism markers, we explored the association of iFGF-23, iFGF-23/25(OH)D ratio, and Pi with renal and CV outcomes, hospitalizations, and all-cause mortality in univariate analysis. Both iFGF-23 and iFGF-23/25(OH)D ratio were significantly associated with the composite CV outcome (52.27 vs. 78.84, p = 0.037 and 3.54 vs 8.56, p=0.03), but not with CKD progression (52 vs 80.42, p = 0.067; 3.81 vs 6.0, p=0.128), hospitalizations (43 vs 22, p=0.120; 6.0 vs 7.23, p=0.200), all-cause mortality (55.41 vs 97.79, p=0.247; 5.6 vs 5.7, p=0.76) or MACCEs (54 vs. 132, p = 0.120; 5.53 vs 8.00, p=0.896) (Table 5). Similar findings were observed in the analysis of patients without vitamin D supplementation (data not shown). On the other hand, serum Pi was significantly associated with both the composite CV outcome (3.25 vs 3.6, p=0.045) and all-cause mortality (3.3 vs 4.0, p=0.020), in univariate analysis (Table 5). When we carried out a stepwise regression analysis, we found that serum Pi, but not iFGF23, proved to be an independent predictor of death, regardless of age, baseline CV disease, hypertension, diabetes, dyslipidemia, iFGF-23 levels, iPTH and BNP (Table 6). In addition, FGF-23 did not predict the

composite CV outcome after adjusting for the same variables, including Pi levels. We then divided the population in terciles according to serum Pi levels (<3mg/dL; 3-3.6 mg/dL; ≥3.7 mg/dL) and observed a distribution of the fatality of 0%, 20%, and 80%, respectively, in the three groups (p=0.034). Detailed analysis showed that among patients who died, serum Pi levels ranged from 3.0 to 4.4 mg/dL.

4. Discussion

In the present study, we examined, in a non-dialysis CKD population under outpatient nephrological care, with renal function spanning all five CKD stages, the associations between the serum levels of iFGF-23 and Pi with CV disease biomarkers and explored prospectively their role as predictors of adverse events and mortality in a 5-year follow-up period. In adjusted models, only serum Pi (independent of other confounders except GFR) remained significantly associated with all-cause mortality. In unadjusted analysis, we found that both iFGF-23 and Pi levels were associated with baseline CV risk factors as well as with a composite CV outcome during follow-up. However, in adjusted analysis, we found that serum Pi levels, but not iFGF-23 were associated with mortality among CKD patients, independent of other confounding factors except GFR. Segmentation of the population into terciles according to serum Pi levels at baseline showed a significant increase in mortality during follow-up among patients with serum Pi levels within the high-"normal" range but not in patients with serum Pi levels below "normal" targets. These findings reinforce the view that high-normal Pi concentrations may carry clinical significance in CKD and are supported by previous studies in both the general population (18) and non-dialysis CKD cohorts (19). They also suggest that current reference values for serum Pi in non-dialysis CKD patients may benefit from re-evaluation (20).

Several mechanisms may underlie the pathogenicity of elevated serum Pi in CKD, including promotion of vascular calcification via osteoblastic transformation of vascular smooth muscle cells and calcium phosphate deposition (21),(22), LVH and myocardial fibrosis (23) and statin-resistance due to altered lipid metabolism (24). Indeed, a secondary analysis of the AURORA trial revealed that higher levels of serum Pi may blunt the CV benefit of statin treatment in dialysis patients (24). Endothelial dysfunction has also been implicated in phosphate's pathogenicity. Translational models have shown that Pi loading can impair endothelial function, likely through disruption of nitric oxide signalling (25). Stevens et al. showed that sustained oral Pi loading in 19 healthy volunteers caused endothelial dysfunction, which was accompanied by significant increases in serum FGF-23 and urinary Pi excretion, without significant changes in serum Pi levels (26). Recent data confirm that glycerol-3 phosphate (G3P), secreted by the proximal renal tubular cells, increases in response to Pi loading, with a subsequent increase in FGF-23 before serum Pi concentration rises (27). The deleterious influence of Pi load on endothelial function may be reversible and assume relevance in

the CKD population, in which the compromised renal excretion of ingested Pi is counteracted by an increase in FGF-23 levels from the earliest CKD stages. We previously reported in a group of CKD patients with a mean GFR of 49 ml/min that the restriction of Pi intake for 14 days from a baseline intake of 1100 mg/day to 700 mg/day was accompanied by a significant improvement in endothelial function, going along with non-significant reductions in FGF-23, iPTH, and Pi levels (28).

As shown in our cohort, the FEp increased significantly with declining renal function, consistent with compensatory responses to early Pi retention. This increase may reflect enhanced FGF–23–mediated phosphaturia in the setting of reduced nephron mass. However, despite this adaptation, serum Pi levels still predicted mortality, and prior studies have demonstrated that heart failure and CV mortality are independent of FEp, in patients with moderate to advanced stages of CKD (29). Therefore, we consider that Pi pathogenicity begins early, even at levels deemed normal, and that its management should be anticipated to prevent CV complications.

In the present study iFGF-23 levels increased with declining renal function and were significantly associated with CV disease and risk factors at baseline, thus reinforcing the role of FGF-23 as an indicator of established CV disease in CKD (30) (31). However, iFGF-23 levels were not independently associated with the composite CV outcome or mortality during follow-up. This is in line with findings from previous studies (32)(33)(34), and reinforces the view that iFGF-23 may function more as a biomarker of disease burden than as a causal factor (35). Indeed, many controversies exist regarding FGF-23 levels and its role as a predictor versus biomarker of CV disease (32). Elevated FGF-23 levels are not specific to CKD and were also associated with various off-target effects on multiple organs, including fractures (36), infections (37), inflammation (38), hereditary hypophosphatemic rickets (39) and even prostate cancer (40). These associations across a wide range of disease states could reflect the pleiotropy of FGF-23 in disease causation. Hereditary disorders of FGF-23 excess share a common biochemical phenotype, including low Pi and normal or low-1,25-(OH)VitD that can be treated with a human monoclonal antibody targeted against FGF-23. Notably, it has been reported that treatment with an anti-FGF-23 monoclonal antibody, although correcting rickets, increased serum Pi to the lower end of the normal range and elevated $1,25(OH)_2D$ levels, was also associated with the development of cardiac calcification (41,42).

In this study, a significant proportion of our cohort received vitamin D supplementation, which may influence FGF-23 levels (43). Moreover, other authors have reported that elevated FGF-23 combined with low vitamin D were associated with adverse outcomes (44). Notably, subgroup analysis showed consistent results across treated and untreated groups. As with iFGF-23, the iFGF-23/25(OH)D ratio was also associated with the composite CV outcome only in univariate analysis, however, we acknowledge that FGF-23 more strongly influences $1,25(OH)_2D$ than 25(OH)D levels, and that the generally adequate 25(OH)D levels in our cohort may help explain the absence of significant effects. Towards a deeper understanding of the pathophysiological role of FGF-23 in CKD-associated CV disease, further mechanistic studies may be warranted.

Overall, these findings reinforce the view that early elevation of FGF-23—even in normophosphatemic patients—may represent the impaired ability to maintain Pi balance. This "maladaptive" increase may help identify high-risk individuals who might benefit from intensified therapeutic strategies targeting Pi control (45). Parallel to this, a lower target for Pi levels may warrant additional benefit regarding CV outcomes, particularly in CKD.

We acknowledge some limitations of our study. First, it is a single-center study with a relatively small number of patients and outcomes; second, the results in our ethnically homogeneous population may not be generalizable to other ethnic groups; third, the "snapshot" evaluation of Pi and FGF-23 levels at baseline could underestimate the association with the outcomes during follow-up.

Our study has also strengths that should be emphasized: First, its prospective nature and the significant follow-up period of nearly five years; second, the inclusion of patients from both genders and spanning all five CKD stages; third, the assessment of the intact biologically active isoform of FGF-23 rather than the inactive C-terminal FGF-23 fragment. The data presented here offers valuable preliminary insights that can serve as a foundation for future, larger-scale and more targeted investigations.

In conclusion, our results agree with the view that serum Pi levels within the high-normal range, are a risk predictor for mortality in non-dialysis CKD patients. Our results also suggest that the contribution of iFGF-23 as a biomarker to CV outcomes and mortality in CKD may not outweigh the role of serum Pi levels within the high-"normal" range. Because serum Pi is a biomarker easily affordable, our findings reinforce the

need to re-examine contemporary guidelines for serum Pi reference levels in the non-dialysis CKD population. This may allow the establishment of more timely interventions, whether through dietary adjustments or pharmacological measures, aimed at enhancing overall Clinical Outcomes in the CKD Population.

Ética de la publicación

1. ¿Su trabajo ha comportado experimentación en animales?:

No

2. ¿En su trabajo intervienen pacientes o sujetos humanos?:

Sí

Si la respuesta es afirmativa, por favor, mencione el comité ético que aprobó la investigación y

el número de registro.:

The research was approved by the Ethics Committee for Health and the Local Institutional Review Board of São João University Hospital Centre (CES 251.14) and

was carried out in accordance with the Declaration of Helsinki (2008) of the World

Medical Association.

Si la respuesta es afirmativa, por favor, confirme que los autores han cumplido las normas

éticas relevantes para la publicación. :

Sí

Si la respuesta es afirmativa, por favor, confirme que los autores cuentan con el

consentimiento informado de los pacientes. :

Sí

3. ¿Su trabajo incluye un ensayo clínico?:

No

4. ¿Todos los datos mostrados en las figuras y tablas incluidas en el manuscrito se recogen en el

apartado de resultados y las conclusiones?:

Sí

Declaration of Conflicting Interests: All the authors declare that there is no conflict of interest.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Author Contributions:

Conceptualization, A.C., J.Q.-S. and M.P.; methodology, A.C., J.Q.-S., N.P; software, A.C., J.Q.-S., and C.C.D.; validation, A.C., J.Q.-S. and C.C.D.; formal analysis, A.C., J.Q.-S. and C.C.D.; investigation, A.C., J.Q.-S., N.P.; C.C.D. and M.P.; resources, A.C., J.Q.-S., N.P.; C.C.D. and M.P.; data curation, A.C., J.Q.-S., N.P. and C.C.D.; writing—original draft preparation, A.C., J.Q.-S., and M.P.; writing—review and editing, A.C., J.Q.-S. and M.P.; visualization, A.C., J.Q.-S., N.P.; C.C.D. and M.P.; supervision, M.P. and J.Q.-S.; project administration, J.Q.-S. and M.P.; funding acquisition, M.P., J.Q.-S. and A.C.

All authors have read and agreed to the published version of the manuscript.

Funding: This work was financed by FEDER-Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020-Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through FCT-Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project “Institute for Research and Innovation in Health Sciences” (POCI-01-0145-FEDER-007274), and a grant from Portuguese Society of Nephrology.

Acknowledgements: This article is a result of the project NORTE-01-0145-FEDER-000012, supported

by Norte Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

References:

1. Jankowski J, Floege J, Fliser D, Böhm M, Marx N. Cardiovascular Disease in Chronic Kidney Disease: Pathophysiological Insights and Therapeutic Options. Circulation. 2021 Mar 16;143(11):1157–72.

2. Bikbov B, Purcell CA, Levey AS, Smith M, Abdoli A, Abebe M, et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2020 Feb 29;395(10225):709–33.

3. Marando M, Tamburello A, Salera D, Di Lullo L, Bellasi A. Phosphorous metabolism and manipulation in chronic kidney disease. Nephrology. 2024 Dec 1;29(12):791–800.

4. Ketteler M, Evenepoel P, Holden RM, Isakova T, Jørgensen HS, Komaba H, et al. Chronic kidney disease–mineral and bone disorder: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2025 Mar 1;107(3):405–23.

5. Figurek A, Rroji M, Spasovski G. FGF23 in Chronic Kidney Disease: Bridging the Heart and Anemia. Cells. 2023;12(4).

6. Bellasi A, Di Micco L, Russo D, De Simone E, Di Iorio M, Vigilante R, et al. Fractional Excretion of Phosphate (FeP) Is Associated with End-Stage Renal Disease Patients with CKD 3b and 5. J Clin Med. 2019 Jul 12;8(7):1026.

7. Magagnoli L, Ciceri ,Paola, and Cozzolino M. Secondary hyperparathyroidism in chronic kidney disease: pathophysiology, current treatments and investigational drugs. Expert Opin Investig Drugs. 2024 Aug 2;33(8):775–89.

8. 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 Adv. 2024 Jan 1;3(1):100747.

9. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. Kidney Int. 2017 Jul 1;92(1):26–36.

10. Evenepoel P, Opdebeeck B, David K, D'Haese PC. Bone-Vascular Axis in Chronic Kidney Disease. Adv Chronic Kidney Dis. 2019 Nov 1;26(6):472–83.

11. Bellasi A, Mandreoli M, Baldrati L, Corradini M, Di Nicolò P, Malmusi G, et al. Chronic Kidney Disease Progression and Outcome According to Serum Phosphorus in Mild-to-Moderate Kidney Dysfunction. Clin J Am Soc Nephrol. 2011 Apr;6(4):883–91.

12. Da J, Xie X, Wolf M, Disthabanchong S, Wang J, Zha Y, et al. Serum Phosphorus and Progression of CKD and Mortality: A Meta-analysis of Cohort Studies. Am J Kidney Dis. 2015 Aug;66(2):258–65.

13. Nakano T, Kishimoto H, Tokumoto M. Direct and indirect effects of fibroblast growth factor 23 on the heart. Front Endocrinol [Internet]. 2023;Volume 14-2023. Available from: https://www.frontiersin.org/journals/endocrinology/articles/10.3389/fendo.2023.1059179

14. Eddington H, Hoefield R, Sinha S, Chrysochou C, Lane B, Foley RN, et al. Serum Phosphate and Mortality in Patients with Chronic Kidney Disease. Clin J Am Soc Nephrol [Internet]. 2010;5(12). Available from: https://journals.lww.com/cjasn/fulltext/2010/12000/serum_phosphate_and_mortality_in_patients_with.18.aspx

15. Gutiérrez Orlando M., Mannstadt Michael, Isakova Tamara, Rauh-Hain Jose Alejandro, Tamez Hector, Shah Anand, et al. Fibroblast Growth Factor 23 and Mortality among Patients Undergoing Hemodialysis. N Engl J Med. 359(6):584–92.

16. 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 Adv. 2024 Jan 1;3(1):100747.

17. Stevens PE, Ahmed SB, Carrero JJ, Foster B, Francis A, Hall RK, et al. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int. 2024 Apr 1;105(4):S117–314.

18. Mendonça L, Gonçalves F, Sampaio S, Castro-Chaves P, Pereira L. Association between serum phosphorus and mortality in NHANES 2003–2006: the effect of gender and renal function. J Nephrol. 2022 Jan;35(1):165–78.

19. Fan Z, Li R, Pan M, Jiang Y, Li Y, Liu L, et al. Relationship between serum phosphorus and mortality in non-dialysis chronic kidney disease patients: evidence from NHANES 2001–2018. BMC Nephrol. 2024 Mar 6;25(1):89.

20. Eddington H, Hoefield R, Sinha S, Chrysochou C, Lane B, Foley RN, et al. Serum Phosphate and Mortality in Patients with Chronic Kidney Disease. Clin J Am Soc Nephrol. 2010 Dec;5(12):2251–7.

21. Kutikhin AG, Feenstra L, Kostyunin AE, Yuzhalin AE, Hillebrands JL, Krenning G. Calciprotein Particles. Arterioscler Thromb Vasc Biol. 2021 May 5;41(5):1607–24.

22. Kuro-o M. Phosphate as a Pathogen of Arteriosclerosis and Aging. J Atheroscler Thromb. 2021 Mar 1;28(3):203–13.

23. Ogata H, Sugawara H, Yamamoto M, Ito H. Phosphate and Coronary Artery Disease in Patients with Chronic Kidney Disease. J Atheroscler Thromb. 2024 Jan 1;31(1):1–14.

24. Massy ZA, Merkling T, Wagner S, Girerd N, Essig M, Wanner C, et al. Association of Serum Phosphate with Efficacy of Statin Therapy in Hemodialysis Patients. Clin J Am Soc Nephrol [Internet]. 2022;17(4). Available from: https://journals.lww.com/cjasn/fulltext/2022/04000/association_of_serum_phosphate_with_efficacy_of.12.aspx

25. Stevens KK, Denby L, Patel RK, Mark PB, Kettlewell S, Smith GL, et al. Deleterious effects of phosphate on vascular and endothelial function via disruption to the nitric oxide pathway. Nephrol Dial Transplant. 2016 Jul 21;gfw252.

26. Gerber JS, Arroyo EMP, Pastor J, Correia M, Rudloff S, Moe OW, et al. Controlled dietary phosphate loading in healthy young men elevates plasma phosphate and FGF23 levels. Pflüg Arch - Eur J Physiol. 2025 Mar 1;477(3):495–508.

27. Zhou C, Shi Z, Ouyang N, Ruan X. Hyperphosphatemia and Cardiovascular Disease. Front Cell Dev Biol. 2021 Mar 4;9:644363.

28. Cerqueira A, Cristino L, Quelhas Santos J, Correia F, Pestana M. SP388THE DECREASE IN PHOSPHATE INTAKE IMPROVES ENDOTHELIAL FUNCTION IN PRE-DIALYSIS CKD PATIENTS. Nephrol Dial Transplant. 2018 May 1;33(suppl_1):i477–i477.

29. Mendonça L, Bigotte Vieira M, Neves JS. Association of combined fractional excretion of phosphate and FGF23 with heart failure and cardiovascular events in moderate and advanced renal disease. J Nephrol. 2023 Jan 1;36(1):55–67.

30. Fuchs MAA, Burke EJ, Latic N, Murray SL, Li H, Sparks MA, et al. Fibroblast growth factor 23 and fibroblast growth factor receptor 4 promote cardiac metabolic remodeling in chronic kidney disease. Kidney Int. 2025 May 1;107(5):852–68.

31. Kishimoto H, Nakano T, Torisu K, Tokumoto M, Uchida Y, Yamada S, et al. Indoxyl sulfate induces left ventricular hypertrophy via the AhR-FGF23-FGFR4 signaling pathway. Front Cardiovasc Med [Internet]. 2023;Volume 10-2023. Available from: https://www.frontiersin.org/journals/cardiovascular-medicine/articles/10.3389/fcvm.2023.990422

32. Bao JF, Hu PP, She QY, Li A. A Land of Controversy: Fibroblast Growth Factor-23 and Uremic Cardiac Hypertrophy. J Am Soc Nephrol. 2020 Jul;31(7):1423–34.

33. Shardlow A, McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Associations of fibroblast growth factor 23, vitamin D and parathyroid hormone with 5-year outcomes in a prospective primary care cohort of people with chronic kidney disease stage 3. BMJ Open. 2017 Aug 1;7(8):e016528.

34. Yeung WCG, Toussaint ND, Lioufas N, Hawley CM, Pascoe EM, Elder GJ, et al. Vitamin D status and intermediate vascular and bone outcomes in chronic kidney disease: a secondary post hoc analysis of IMPROVE-CKD. Intern Med J. 2024 Dec 1;54(12):1960–9.

35. Stöhr R, Schuh A, Heine GH, Brandenburg V. FGF23 in Cardiovascular Disease: Innocent Bystander or Active Mediator? Front Endocrinol. 2018 Jun 27;9:351.

36. Mirza MA, Karlsson MK, Mellström D, Orwoll E, Ohlsson C, Ljunggren Ö, et al. Serum fibroblast growth factor-23 (FGF-23) and fracture risk in elderly men. J Bone Miner Res. 2011 Apr 1;26(4):857–64.

37. Nowak KL, Bartz TM, Dalrymple L, de Boer IH, Kestenbaum B, Shlipak MG, et al. Fibroblast Growth Factor 23 and the Risk of Infection-Related Hospitalization in Older Adults. J Am Soc Nephrol [Internet]. 2017;28(4). Available from: https://journals.lww.com/jasn/fulltext/2017/04000/fibroblast_growth_factor_23_and_the_risk_of.26.aspx

38. Rossaint J, Unruh M, Zarbock A. Fibroblast growth factor 23 actions in inflammation: a key factor in CKD outcomes. Nephrol Dial Transplant. 2017;32(9):1448–53.

39. Pastor-Arroyo EM, Gehring N, Krudewig C, Costantino S, Bettoni C, Knöpfel T, et al. The elevation of circulating fibroblast growth factor 23 without kidney disease does not increase cardiovascular disease risk. Kidney Int. 2018;94(1):49–59.

40. Feng S, Wang J, Zhang Y, Creighton CJ, Ittmann M. FGF23 promotes prostate cancer progression. Oncotarget. 2015;6(19):17291.

41. Shalhoub V, Shatzen EM, Ward SC, Davis J, Stevens J, Bi V, et al. FGF23 neutralization improves chronic kidney disease - Associated hyperparathyroidism yet increases mortality. J Clin Invest. 2012;122(7):2543–53.

42. Bacchetta J, Bardet C, Prié D. Physiology of FGF23 and overview of genetic diseases associated with renal phosphate wasting. X-Linked Hypophosphatemia. 2020 Feb 1;103:153865.

43. Charoenngam N, Rujirachun P, Holick MF, Ungprasert P. Oral vitamin D3 supplementation increases serum fibroblast growth factor 23 concentration in vitamin D-deficient patients: a systematic review and meta-analysis. Osteoporos Int. 2019 Nov;30(11):2183–93.

44. Mitroi C, Rivas-Lasarte M, Hernández-Pérez FJ, Gómez-Bueno M. La vitamina D en la insuficiencia cardiaca: realidades y esperanzas. Rev Esp Cardiol. 2022;22:1–20.

45. González-Casaus ML, Gonzalez-Parra E, Fernandez-Calle P, Buño-Soto A. FGF23: de la nefrología de salón a la cabecera del paciente. Nefrología. 2021 May 1;41(3):276–83.

Table 1. Characterization of the non-dialysis patient population by CKD stages (n=82).

	Stage 1-2 (n=29)	Stage 3a -3b (n=25)	Stage 4 -5 (n=28)	p[1]	p* [1-2 vs 3a-3b]	p* [1-2 vs 4-5]	p* [3a-3b vs 4-5]
DEMOGRAFIC DATA							
Age (years), mean±sd	49.2 ±14.3	58.0 ±15.6	66.3 ±13.7	<0.001	0.083	<0.001	0.125
Gender Male (n, %)	11 (37.9)	17 (68.0)	14 (50.0)	0.087			
Body mass index mean±sd	29.4 ±6.8	28.9 ±4.5	25.5 ±4.9	0.075			
Cardiovascular disease, n (%)	4 (13.8)	9 (36.0)	10 (35.7)	0.104			
Charlson Index score mean±sd	1.6 ±2.1	4.4 ±2.9	6.1 ±2.4	<0.001	<0.001	<0.001	0.037
Diabetes, n (%)	5 (13.8)	8 (32.0)	14 (50.0)	0.013[3]	0,327	0,009	0,552
Hypertension n (%)	16 (52.2	20 (80.0)	26 (92.9)	0.003[3]	0,162	0,003	0,702
CKD RELATED PARAMETERS							
eGFR CKD-EPI (ml/min/1,73m^2) mean±sd	97.7 ±24.1	44.5 ±7.9	19.9 ±7.0	<0.001	<0.001	<0.001	<0.001
Protein/creatinine ratio (mg/g), median (IQR)	221.0 (84.0-841.6)	402.0 (152.0-1035.4)	981.4 (453.0-2966.0)	0.004[2]	0.336	0.006	0.123
iFGF-23, median (IQR)	28,3 (14.1-54,6)	67,8 (46.2-91,5)	145,9 (98.1-219,9)	<0,001	<0,001	<0,001	<0,001
25(OH)D) (ng/mL), median (IQR)	22 (14-28)	22 (7-31)	24 (14-40)	0.874			
Ratio iFGF-23 / 25(OH)D, Median (IQR)	4.2 (1.9-6.5)	4.1 (1.2-6.8)	7.96 (4.6-8.9)	0.207			
Phosphate (mg/dL) mean±sd	3.0 ±0.4	3.3 ±0.5	3.9 ±0.6	<0.001	0.118	<0.001	<0.001
Fraccional excretion of phosphate (%), mean±sd	16.9±8.8	34.2±15.6	40.1±12.9	0.010	0.010	0.001	0.325
Calcium (mg/dL) mean±sd	4.6 ±0.2	4.8 ±0.3	4.5 ±0.3	0.013	0.222	0.604	0.010
Magnesium (mg/dL), mean±sd	1.62±0.2	1.71±0.18	1.72±0.23	0.319			
Parathormone (pg/mL) mean±sd	53.1±24.6	75.5±39.3	183.6 ±158.0	<0.001	>0.999	<0.001	0.001
CARDIOVASCULAR RELATED PARAMETERS							
BNP (pg/mL) median (IQR)	30.2 (18.3-70.1)	74.8 (67.0-105.0)	74.5 (45.4-327.8)	0.030[2]	0.189	0.036	>0.999
Left Ventricular Mass (g) mean±sd	269.5 ±163.5	175.0 ±72.6	189.5 ±61.2	0.333			
Ejection Fraction (%) mean±sd	60 ±9	62 ±8	61 ±8	0.921			
THERAPEUTICS							
Cholecalciferol supplementation dosis (UI/day), mean±sd	1500 ±503	1467 ±611	1500 ±500	0,927			
Vitamine D analogues, n (%)	1 (3.4)	5 (20.0)	13 (46.4)	<0.001	0.235	<0.001	0.240
Vitamine D analogues dosis (mcg/day), median	0.11	0.11	0.16	ns			

[1]T teste for independent sample; [2]Mann-Whitney test; [3]Chi-Square test; * Bonferroni test. iFGF.23: intact FGF-23; 25(OH)D: 25-hydroxyvitamin D; BNP: B-type natriuretic peptide.

Table 2. – Associations between iFGF-23 and phosphate levels with patients' demographic characteristics and comorbidities.

	iFGF-23			Serum Phosphate		
	Median	*p*	r	Median	*p*	r
Gender (M/F)	(59.9/72.5)	0,492*		(3.40/3.35)	0,599*	
Age			0,460 (<0.001)‡			0.292 (0,008) ‡
Hypertension	79.63	0,005*		3.40	0,027*	
Diabetes	115.43	<0,001*		3.65	0,009*	
Dyslipidemia	78.32	0,075*		3.40	0,358*	
Baseline CV disease	92.00	0,02*		3.50	0,137*	
Baseline CbV disease	121.48	0,146*		3.40	0,601*	
CCI			0,595 (<0.001)‡			0.417 (<0.001)‡

*Mann-Whitney test and ‡Spearman correlation (significance to p-value <0.05); iFGF-23: intact FGF-23; CV – cardiovascular disease; CbV – cerebrovascular disease; CCI – Charlson comorbidity index.

Table 3. Correlation between iFGF-23 and serum phosphate levels, with clinical variables in non-dialysis CKD patients (n=82).

	Circulating intact FGF-23	Serum Phosphate
CKD RELATED PARAMETERS		
eGFR CKD-EPI (ml/min/1,73m^2)	-0,789 (<0.001)	-0.621 (<0.001)
Protein/creatinine ratio (mg/g)	0,297 (0.010)	0.376 (<0.001)
Serum phosporus (mg/dL)	0,558 (<0.001)	--
iFGF-23 (mg/mL)	--	0,558 (<0.001)
Parathormone (pg/mL)	0,595 (<0.001)	0.506 (<0.001)
25-OH-Vitamin D (ng/mL)	0,142 (0,260)	0.024 (0.852)
iFGF-23/25(OH)D ratio	--	0.344 (<0.001)
CARDIOVASCULAR RELATED PARAMETERS		
BNP (pg/mL)	0,312 (0,042)	0.313 (0.041)
Left Ventricular Hypertrophy	-0,152 (0,674)	0.128 (0.724)
Ejection Fraction	0,073 (0,760)	0.69 .772)

Spearman correlation (significance to p-value <0.05); eGFR: estimated glomerular filtration rate; i-FGF-23: intact FGF-23; 25(OH)D: 25-hydroxyvitamin D; BNP: B-type natriuretic peptide.

Table 4. Cardiovascular and renal outcomes upon follow-up [mean 58months (IQ 30-69)] in the studied population.

Outcome (n = 65)	(n, %)
MACCEs	4 (6,2)
Acute myocardial infarction	1 (1,5)
Stroke	1 (1,5)
Composite outcome on CKD progression*[1]	19 (29,2)
Progression to ESRD	10 (15,4)
Hospitalizations	22 (33,8)
All-cause mortality	5 (8)
CV mortality	2 (3)
Composite cardiovascular outcome *	23 (35,4)

MACCEs: major adverse cardiovascular and cerebrovascular events; ESRD: End Stage Renal Disease; CV: cardiovascular.
*Composite cardiovascular outcome: MACCEs, hospitalizations for medical reasons and all-cause mortality.

Table 5. – Association between iFGF-23, ratio iFGF-23/25(OH)D, and phosphate with mortality and renal and cardiovascular outcomes during follow-up in the studied population.

	iFGF-23		Ratio iFGF-23/ 25(OH)D		Serum Phosphate	
	Median	*p*	Median	*p*	Median	*p*
CKD Progression (n=19)	80.42	0.067	6.0	0.128	3.50	0,077
All-cause mortality (n=5)	97.79	0.247	5.7	0.760	4.40	0.020
Hospitalizations (n=22)	78.58	0.095	7.23	0.200	3.60	0.100
MACCES (n=4)	132.53	0.120	8.0	0.896	3.70	0.203
Composite Cardiovascular Outcome* (n=23)	78.84	0.037	8.56	0.03	3.25	0.045

*Composite cardiovascular outcome: MACCEs, hospitalizations for medical reasons and all-cause mortality. iFGF-23: intact FGF-23; 25(OH)D: 25-hydroxyvitamin D.

Table 6. Association between mortality and phosphate, adjusted to renal function and other CV risk factors (logistic regression).

	Mortality		
	OR	IC 95%	p
Model 1			
PHOSPHATE	6,137	0,809-46,573	0,079
Renal function	0,994	0,953-1,038	0,796
Model 2			
PHOSPHATE	6,244	1,067-36,543	0,042
Age	1,048	0,967-1,136	0,253
Model 3			
PHOSPHATE	5,948	0,981-36,062	0,052
Charlson comorbidity index	1,436	0,992-2,080	0,055
Model 4			
PHOSPHATE	7,577	1,0403-40,909	0,019
Cardiovascular disease	1,906	0,250-14,545	0,534
Model 5			
PHOSPHATE	10,943	1,727-69,363	0,011
Hypertension	0,170	0,016-1,818	0,143
Model 6			
PHOSPHATE	7,188	1,375-37,563	0,019
Diabetes	1,174	0,158-8,733	0,876
Model 7			
PHOSPHATE	8,361	1,390-50,295	0,020
Dyslipidemia	0,587	0,069-4,981	0,0625
Model 8			
PHOSPHATE	5,383	1,026-33,220	0,047
iFGF-23	1,002	0,998-1,005	0,384
Model 9			
PHOSPHATE	15.024	1.033-218.459	0.047
iPTH	0.997	0.989-1.005	0.471
Model 10			
PHOSPHATE	6,015	1,042-34,729	0,045
BNP	1,003	0,996-1,010	0,397

iFGF-23: intact FGF-23; iPTH – parathormone; hemoglobin; BNP: B natriuretic peptide.

Point-by-Point Response to Reviewer 1

We sincerely appreciate the reviewer's insightful comments, which have significantly contributed to improving the quality of our manuscript. Below, we address each point in detail and outline the corresponding modifications made to the manuscript.

As main problems I find:

a) Poorly finished: Especially the abstract and bibliography

- We acknowledge and appreciate the comment, and we have improved the abstract according to the structure required by Nefrología (Lines 32-53). Also, the bibliography was updated and formatted according to Nefrología's requirements (Pages 15-17).

b) Very small sample size, especially in patients with GFR <60. With many unresolved confounding variables that make the results hard to believe

We acknowledge the limited sample size, particularly among patients with a GFR <60 mL/min/1.73 m², as we stated in the manuscript as a study limitation. Although it is currently not feasible to expand this cohort, we believe the data presented here offers valuable preliminary insights that can serve as a foundation for future, larger-scale and more targeted investigations (Lines 263-271).

It does not answer the fundamental question: Is FGF23 related to mortality independently of phosphorus?

We thank the reviewer for this insightful comment. As presented in Table 5 (Page 20), our analysis demonstrated that iFGF-23 levels were not significantly associated with either all-cause mortality or the composite cardiovascular outcome. In this setting, no additional analysis was deemed necessary. However, we underline in the results section that FGF-23 did not predict the composite CV outcome after adjusting for the same variables, including phosphate levels (Lines 190-191).

On the contrary, as shown in Table 6 (Page 20), serum phosphate levels independently predicted mortality, irrespective of FGF-23 concentrations.

b) Tables are too large, with intense information that is not used

We appreciate the reviewer's suggestion and have accordingly reduced the content of the tables, aiming to improve clarity and facilitate interpretation (Pages 18-20).

e) Outdated bibliography

- We sincerely thank the reviewer for this observation. In response, we have thoroughly revised and updated the bibliography to include more recent and relevant references. These updated citations reflect current evidence and enhance the scientific rigor of our introduction and discussion. All newly added references are now cited appropriately throughout the manuscript and are listed in the revised reference section according to the journal's formatting guidelines (Pages 15-17).

f) It is true that the authors have made an effort to respond to the referees and have provided interesting data such as the phosphorus excretion index and FGF23/vitamin D ratio, but no information is drawn from these in the discussion.

- We appreciate the reviewer's insightful comment. As noted, a considerable proportion of our cohort, particularly those with more advanced CKD—received vitamin D analogues. This has now been explicitly stated in the Methods and Results sections. Although the administered doses were relatively modest, we conducted stratified analyses based on vitamin D analogue use. These additional analyses revealed that the association between the iFGF-23/25(OH)D ratio and clinical outcomes remained consistent regardless of treatment status. This has been incorporated into the revised manuscript to clarify that the use of vitamin D analogues did not substantially impact our findings (Lines 157-160; 187–194; 239–244).

ABSTRACT

Does not follow the structure required by Nefrología

- The abstract was improved according to the structure required by Nefrología (Lines 32-54).

INTRODUCTION

Citation 3 is repeated.

- The repetition of citation 3 was deleted.

Insufficient references. There are more recent references.

- The bibliography was updated and formatted according to Nefrología's requirements (Pages 15-17).

The study is not well justified:

To better justify the study, we revised the introduction section (lines 66 – 82).

It poses this question in the introduction that it does not answer: Although hyperphosphatemia and FGF-23 are associated with increased mortality in CKD [7], it is not clear whether compensatory increases in FGF-23 secretion induce or protect against the increased cardiovascular morbidity and mortality observed in CKD.

We thank the reviewer for this insightful observation. We acknowledge that the manuscript raises a question in the introduction regarding whether FGF-23 plays a pathogenic role or serves as a compensatory response in the context of cardiovascular morbidity and mortality in CKD—an issue that was not resolved by our study. To address this, we have revised the text to clarify that our findings do not support a direct association between FGF-23 levels and mortality or cardiovascular endpoints. Our data suggest that, although FGF-23 is associated with certain cardiovascular risk factors, we could not demonstrate an association with adverse outcomes. Rather, FGF-23 may reflect the phosphate burden rather than mediating cardiovascular pathology. We have added this interpretation to the Discussion section and emphasised the need for mechanistic studies to further elucidate the role of FGF-23 in CKD-related CVD (Lines 250-258).

- RESULTS

Stage 1 and 2 patients with an average GFR of over 90 ml/min are included as chronic kidney disease. All with MA >30 mg/g. Without them, the patient sample is 53. Having proteinuria can increase FGF23 by lowering klotho, but this would only serve as a control.

We sincerely thank the reviewer for this thoughtful and constructive comment. As noted, our study included patients with CKD stages 1 and 2 who presented with normal or near-normal eGFR but had persistent albuminuria (MA >30 mg/g). Their inclusion was guided by the KDIGO definition of CKD, which incorporates both decreased GFR and the presence of structural or functional markers of kidney damage—albuminuria being one of the most clinically relevant. This information is now included in the Methods section (Lines 88-91). Moreover, studies have shown that FGF-23 levels increase as early as CKD stages 1 and 2 (Salera et al., 2025; Isakova et al., 2011). This rise occurs even when serum phosphate levels remain within the reference range, indicating that FGF-23 acts as an early compensatory mechanism to maintain phosphate homeostasis as kidney function declines. On the other hand, we recognise that proteinuria may contribute to elevated FGF-23 levels, potentially through reduced renal Klotho expression and tubular injury. However, in our cohort, albuminuria was not limited to early CKD stages. Increased levels of proteinuria were observed progressively across advancing CKD stages, consistent with the natural course of disease. Therefore, the potential influence of proteinuria on FGF-23 is not restricted to patients with preserved GFR but rather represents a continuous variable across the spectrum of CKD.

Given the observational nature of our study and our aim to evaluate the relationship of FGF-23 and phosphate with cardiovascular outcomes in a real-world CKD outpatient population, we believe that including stages 1 and 2 patients with albuminuria enhances the clinical applicability of our findings and the potential benefit for earlier interventions. To clarify this point, we have added a statement to the Results section (Lines 147-148).

Numbers are sometimes written out and other times in digits

We thank the reviewer for this observation. We have carefully revised the manuscript to ensure consistency in the formatting of numerals.

There were 15 patients on vitamin D analogues, whose levels are not readable and may influence the results, especially in more advanced CKD

We thank the reviewer for this valuable observation. Indeed, several patients in our cohort were receiving vitamin D analogues, predominantly among those with more advanced CKD stages. However, as noted in the

manuscript, the prescribed doses were low, which we believe has minimised their overall impact on FGF-23 levels. Importantly, we analysed the iFGF-23/25(OH)D ratio and found no significant difference across CKD stages. Furthermore, when evaluating clinical outcomes, the association between iFGF-23 levels and the composite CV endpoint did not change when stratifying patients by vitamin D analogue use, suggesting this treatment did not significantly influence the observed associations. To improve clarity, we have revised the relevant sections of the manuscript to explicitly state the number of patients receiving vitamin D analogues and to address this potential confounder more directly (Lines 156-158; 179-185; 250-257).

iFGF-23/25(OH)D ratio: Many patients were on vitamin D or analogues

We thank the reviewer for this important observation. Indeed, a significant percentage of patients in our cohort were treated with vitamin D analogues, primarily in more advanced CKD stages. We have now clarified this in both the Methods and Results sections. While the prescribed doses were relatively low, we performed additional analyses stratifying patients by vitamin D analogue use. These analyses showed that the iFGF-23/25(OH)D ratio did not significantly differ in its association with clinical outcomes between groups This has been discussed in the revised manuscript to clarify that vitamin D analogue use, in the context of our cohort, did not appear to meaningfully influence the observed findings. Nevertheless, we acknowledge that FGF-23 will likely influence $1,25(OH)_2D$ levels to a much greater extent than 25(OH)D levels and therefore, this consideration has now been incorporated into the revised manuscript (Lines 156-158; 179-185; 250-257).

Tables are too extensive, with information that is later not used

- We thank the reviewer for this helpful observation. In response, we have carefully reviewed the content of the tables and streamlined them to include only variables that are discussed or play a relevant role in the analyses. Redundant or non-essential data has been removed to enhance clarity and improve the focus of the presentation. We believe these revisions contribute to a more concise and reader-friendly manuscript. The updated tables are now aligned more closely with the main text and its objectives (Pages 18-20).

DISCUSSION

Few recent citations. Little discussion about the data provided. FGF23/vitamin D ratio and phosphorus excretion index

We thank the reviewer for these valuable observations. In response, we have revised the discussion to include several recent and relevant references, which provide updated insights into the interplay between FGF-23, phosphate, vitamin D, and cardiovascular risk in CKD. Additional commentary was added regarding the role of the iFGF-23/25(OH)D ratio, noting its association with composite outcomes in univariate analysis but lack of significance in adjusted models. We also expanded our discussion to incorporate the biological rationale behind the fractional excretion of phosphate, which increased across CKD stages in our cohort and may reflect compensatory mechanisms to maintain phosphate homeostasis. These changes aim to improve the depth and contemporaneity of the discussion (Lines 220-222; 228-232; 235-240; 244 -272).

BIBLIOGRAPHY

None of the citations follow the format required by Nefrología. A single author, more than three, unspecified references. They follow no concrete format and not the one required by Nefrología.

We thank the reviewer for highlighting this important formatting issue. In response, we have thoroughly revised all references to adhere strictly to the guidelines of *Nefrología*. These corrections have now been implemented throughout the manuscript and reference section (Pages 14-16)

In conclusion, we sincerely appreciate the reviewer's constructive feedback, which has helped us refine our analysis and improve the clarity of our manuscript. The suggested modifications have been incorporated into the revised version of the manuscript, and we hope these revisions address the reviewer's concerns satisfactorily.

Best regards,

The authors.

Point-by-Point Response to Reviewer 2

We sincerely appreciate the reviewer's insightful and complimentary comments. We fully agree that FGF-23 is likely to influence $1,25(OH)_2D$ levels to a much greater extent than 25(OH)D levels. This important consideration has now been incorporated into the revised manuscript (Lines 253-256).

We sincerely thank the reviewer for the constructive feedback throughout the review process. We are truly grateful for the acceptance of our manuscript in its current form and deeply appreciate the time and effort invested in its evaluation.

Thank you once again for your consideration and support.

Best regards,

The authors.