Research Article

Role of Klotho Deficiency in Mediating Off-Target Effects of Elevated FGF23 in Chronic Kidney Disease: A Clinical and Molecular Correlation Study

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Article Info

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Abstract

Background: The increase of plasma fibroblast growth factor 23 (FGF23) gives rise to pathological cardiovascular effects in chronic kidney disease (CKD) via Klotho-independent off-target receptor activation. In chronic kidney disease (CKD), soluble alpha-Klotho, an essential co-receptor for directing FGF23 signalling to kidneys declines, resulting in its stronger effect at off-target sites. We hypothesized that Klotho deficiency alters the intensity of cardiovascular remodelling induced by FGF23 in a manner that is measurable in a clinical setting.

Methods: This was a cross-sectional study carried out in 160 hospitalised patients with CKD from stages G2–G5 (40 per stage). Serum FGF23 as well as soluble α-Klotho were measured using ELISA. The cardiac restructuring was measured by means of carotid intima-media thickness (CIMT), ankle-brachial pressure index (ABPI), left ventricular hypertrophy (LVH), and aortic valve (AV) sclerosis. Patients were stratified by Klotho tertiles (<37.6, 37.6–69.4, >69.4 pg/mL) and FK (FGF23: Klotho) ratio tertiles. We computed Spearman correlations between log-FGF23 and cardiovascular outcomes in each stratum of Klotho. A comparison of cardiovascular outcomes was made from a quadrant interaction analysis across four axis states, using low/high FGF23 along with low/high Klotho.

Results: Klotho levels were not significantly different between CKD stages (median 48.1-58.2 pg/mL, Kruskal-Wallis p=0.907) and 22.5-32.5% of patients in each stage were severely deficient (<33 p g/mL). Similarly, the FGF23: Klotho ratio increased from G2 (median 1.75) to G5 (4.47; p<0.001), indicating progressive axis uncoupling. The relationship between log-FGF23 and CIMT was significantly influenced by Klotho stratum: r=0.642 (p<0.001) in the low-Klotho tertile; r=0.317 (p=0.021) in the middle tertile; and r=0.047 (p=0.739, not significant) in the high-Klotho tertile. The patients with high FK ratio had

greater CIMT than those with a low FK ratio. The results of the study show that the CIMT significantly increased across FK ratio tertiles. The group of seven patients with high FGF23 and low Klotho (mean FGF23=758 pg/mL, Klotho=23 pg/mL) had a mean CIMT of 1.364±0.069 mm, significantly greater than that of the reference group with low FGF23 and high Klotho (1.065±0.181 mm; p=0.0003). When analyzed independently, there was no significant difference in LVH and AV sclerosis across Klotho strata.

Conclusions: Klotho deficiency is an important permissive factor that enhances the carotid atherosclerotic impact of high FGF23 during CKD. This uncoupling is represented by the FGF23: Klotho ratio, which better correlates to vascular remodelling than either biomarker alone. These findings provide clinical and molecular evidence that Klotho availability halts the magnitude of off-target FGF23 effects and that measures promoting Klotho availability is preferred over FGF23 suppression.

Introduction

The key determinant of renal phosphate handling and 1,25-dihydroxyvitamin D synthesis is the Fibroblast growth factor 23 (FGF23)–α-Klotho axis. Under physiological conditions, the circulating osteocyte-derived hormone FGF23 binds to and activates a ternary complex consisting of fibroblast growth factor receptor 1 (FGFR1) and its obligate co-receptor α-Klotho, which is expressed almost exclusively in renal distal tubule [1, 2]. The Klotho-gated signalling mechanism gives specifies the actions of FGF23, ensuring that the phosphaturic and vitamin D-suppressing actions are limited to the tubular epithelium, preventing off-target actions.

The elegant architecture of the glomerulus is progressively dismantled in chronic kidney disease (CKD). At the earliest stages of renal impairment, even before the emergence of hyperphosphatemia and secondary hyperparathyroidism, levels of FGF23 start to rise exponentially. In end-stage kidney disease (ESKD) for example, they may reach over 1000-fold the normal value [3]. Meanwhile, expression of α-Klotho in the renal tubule is downregulated by uraemic toxins, inflammatory cytokines and via epigenetic changes [4, 5]. Owing to this dual dysregulation, FGF23 progressively uncouples from its renal gatekeeper, clinically quantified by the FGF23: Klotho ratio. As Klotho declines, FGF23 in concentrations that ultimately overwhelm the physiological levels commences activation of alternative, lower-affinity receptors in tissues that do not constitutively express α-Klotho. The fibroblast growth factor receptor 4 (FGFR4) on cardiomyocytes is the most important of these. FGF23, in a strictly Klotho-independent manner, activates the calcineurin–nuclear factor of activated T-cells (NFAT) hypertrophic signalling cascade in cardiomyocytes through this receptor promoting pathological left ventricular hypertrophy (LVH) [6, 7]. In the vasculature, depletion of soluble Klotho, the ectodomain shed from tubular cells into the circulation, removes its inhibition of Na^+/Pi co-transporters in vascular smooth muscle cells (VSMCs). This allows phosphate uptake, osteogenic trans differentiation, and medial calcification [5, 8]. Klotho also normally promotes eNOS via the PI3K/Akt pathway; its loss limits NO bioavailability, leading to endothelial dysfunction and accelerates subclinical atherosclerosis [9]. An important yet overlooked question is whether Klotho deficiency acts simply as a bystander, alongside FGF23 elevation, independently resulting in cardiovascular injury, or as a modifier whose absence exacerbates the cardiovascular toxicity of a raised plasma FGF23 concentration. If Klotho acts as a gate, limiting access of FGF23 to off-target receptors, then lower Klotho levels should correlate with stronger associations between FGF23 concentration and cardiac remodelling outcomes.

The current study aims at addressing this question directly among CKD patients. It therefore assessed a cross-sectional cohort of 160 CKD patients across stages G2–G5, with concurrent measurements of serum FGF23, soluble Klotho, and four parameters of cardiovascular remodelling. Through stratifying FGF23-cardiovascular correlations by Klotho status and assessing the FGF23: Klotho ratio as an index of axis uncoupling, we aimed to provide clinical and molecular evidence for Klotho gating of FGF23 mediated off-target cardiovascular effects.

Methods

Study design and participants

A cross-sectional observational study was conducted at a tertiary care facility. Adult patients who were consecutively admitted in the hospital with certified CKD as per the KDIGO 2012 criteria, having stable renal function and without any intercurrent acute kidney injury were enrolled in the study. A total of 160 participants were included, stratified a priori into four groups of 40 by CKD stage G2 (eGFR 60–89 mL/min/1.73m²), G3 (30–59), G4 (15–29), and G5 (<15 mL/min/1.73m², pre-dialysis). Those patients who underwent renal replacement therapy were excluded. The additional exclusion criteria included active malignancy, acute inflammatory disease, severe hepatic disease and pregnancy. The study employed the CKD-EPI 2021 creatinine equation [10] to estimate GFR. The study was conducted in accordance with the Declaration of Helsinki and Institutional ethics committee approval and written informed consent were obtained from all participants.

Biochemical measurements

All laboratory parameters were measured on fasting conditions: serum creatinine, blood urea nitrogen (BUN), electrolytes, sodium, potassium, chloride, and bicarbonate, haemoglobin, albumin and fasting diabetic profile. The concentration of serum FGF23 was assessed using a C-terminal FGF23 ELISA. As 1000 pg/mL was the upper limit of the assay, any readings above this were recorded as >1000 and for analyses requiring

continuous values were treated as 1000 pg/mL. The quantity of soluble alpha-Klotho was assessed by using the sandwich ELISA assay. Before the correlation analyses, the values for FGF23 were log transformed. We calculated the FGF23: Klotho ratio (FK ratio) by dividing FGF23 (pg/mL) with Klotho (pg/mL) to serve as a continuous index of axis uncoupling. For a comparison of sub-groups, FGF23 and Klotho were divided into tertiles, and the boundaries for FK ratio tertiles were T1/T2: 1.25 and T2/T3: 4.17.

Cardiovascular remodelling assessments

Four cardiovascular remodelling markers were measured. (1) CIMT- Carotid Intima Media Thickness, measured by B-mode ultrasonography, was performed on the far wall of right and left common carotid arteries (1 cm proximal to the carotid bifurcation) to obtain mean of three measurements in each side in mm. A CIMT of over 1.0 mm was regarded as subclinical atherosclerosis and over 1.2 mm as significant atherosclerosis. (2) The ankle-brachial pressure index (ABPI) was measured with a hand-held Doppler probe; ABPI <0.90 was taken as indicative of peripheral arterial disease (PAD), whilst values >1.40 were flagged as potentially non-compressible. (3) Left ventricular hypertrophy (LVH) was defined as echocardiographically left ventricular mass index >115 g/m² in men and >95 g/m² in women based on ASE criteria recorded as binary (yes/no). (4) As per the echocardiography findings, aortic valve sclerosis was identified as valve leaflet thickening or calcification without a haemodynamically significant obstruction. Thus, aortic valve sclerosis was recorded as binary.

Statistical analyses

Continuous variables were examined for distributional characteristics and reported either as mean ± standard deviation when normally distributed or as median with interquartile range when skewed. Categorical data were summarised as counts and percentages. Group comparisons across CKD stages G2–G5 were conducted using the Kruskal–Wallis test for continuous measures and the chi-square test for categorical measures, with Dunn's procedure applied for post-hoc contrasts. Because of non-normality, serum FGF23 concentrations were log-transformed prior to correlation analyses. For continuous analyses, values above the assay detection ceiling of 1000 pg/mL were truncated at that limit. All statistical procedures were implemented in Python version 3.11 using the pandas, scipy, scikit-learn, and statsmodels libraries.

Associations between components of the FGF23–Klotho axis and cardiovascular remodelling indices (carotid intima-media thickness [CIMT], ankle-brachial pressure index [ABPI], left ventricular hypertrophy [LVH], and aortic valve sclerosis) were assessed using Spearman rank correlation. Patients were further stratified by tertiles of circulating Klotho and by tertiles of the FGF23: Klotho ratio to explore the modifying influence of Klotho deficiency on FGF23-related cardiovascular changes. A composite vascular remodelling score was derived by standardising CIMT, inverting ABPI, and incorporating LVH and aortic valve sclerosis status. Interaction analyses were performed to evaluate the combined effect of high/low FGF23 and high/low Klotho status on vascular remodelling. Statistical significance was defined as $p < 0.05$.

Results

Participant characteristics and CKD staging

A total of 160 patients (mean age 59.8±12.7 years; 33.8% female) were included. 66.9% had diabetes mellitus and 65.0% had hypertension. Results stratified by CKD stage are shown in Table 1. eGFR was significantly different between groups (G2: 69.7±12.8; G3: 42.9±9.9; G4: 22.6±6.4; G5: 8.1±3.5 mL/min/1.73m²; H=142.95, p<0.001), confirming appropriate allocation. The G5 patients were significantly younger (51.7±13.1 years) than G2-G4 patients which perhaps indicates an earlier disease onset in this South Asian population or a referral bias.

Table 1: Baseline clinical, biochemical, and cardiovascular characteristics stratified by CKD stage.

Variable	CKD G2 (n=40)	CKD G3 (n=40)	CKD G4 (n=40)	CKD G5 (n=40)	p value
Age (years), mean±SD	63.5±13.0	60.6±11.1	63.5±9.8	51.7±13.1	<0.001
Female sex, n (%)	11 (28)	15 (38)	14 (35)	14 (35)	0.729
Diabetes mellitus, n (%)	25 (63)	27 (68)	33 (83)	22 (55)	0.062
Hypertension, n (%)	23 (58)	21 (53)	29 (73)	31 (78)	0.022
eGFR (mL/min/1.73m²), mean±SD	69.7±12.8	42.9±9.9	22.6±6.4	8.1±3.5	<0.001
Creatinine (mg/dL), mean±SD	1.14±0.24	1.72±0.45	2.93±0.68	10.54±19.4	<0.001
BUN (mg/dL), mean±SD	15.6±7.4	21.5±9.3	38.4±16.1	48.3±21.9	<0.001
Bicarbonate (mmol/L), mean±SD	22.4±2.7	21.3±4.6	18.5±3.1	17.9±4.2	<0.001
Haemoglobin (g/dL), mean±SD	11.7±2.4	10.2±2.0	9.4±1.7	8.6±1.8	<0.001
Albumin (g/dL), mean±SD	3.66±0.65	3.76±0.41	3.25±0.78	3.48±0.63	0.008
SBP (mmHg), mean±SD	133.0±23.1	126.3±16.4	133.0±21.7	143.5±19.0	0.001

Variable	CKD G2 (n=40)	CKD G3 (n=40)	CKD G4 (n=40)	CKD G5 (n=40)	p value
FGF23 (pg/mL), median [IQR]	52.2 [29.9–345.9]	66.3 [29.2–121.2]	129.2 [58.7–495.6]	190.9 [103.5–695.6]	<0.001
FGF23 >1000 pg/mL, n (%)*	6 (15)	4 (10)	16 (40)	14 (35)	<0.001
log FGF23, median [IQR]	3.95 [3.40–5.84]	4.20 [3.37–4.80]	4.86 [4.07–6.19]	5.25 [4.64–6.54]	<0.001
Soluble Klotho (pg/mL), median [IQR]	48.1 [34.2–68.2]	49.5 [28.8–86.9]	55.6 [34.5–82.1]	58.2 [33.6–86.1]	0.907
Klotho <33 pg/mL (severe deficiency), n (%)	9 (23)	13 (33)	9 (23)	10 (25)	0.652
Klotho <50 pg/mL, n (%)	20 (50)	20 (50)	16 (40)	18 (45)	0.726
FGF23:Klotho ratio, median [IQR]	1.75 [0.92–3.98]	1.35 [0.68–2.28]	3.07 [1.24–7.02]	4.47 [1.56–12.53]	<0.001
CIMT (mm), mean±SD	0.898±0.087	1.004±0.084	1.164±0.107	1.283±0.116	<0.001
CIMT >1.2 mm (significant), n (%)	0 (0)	0 (0)	12 (30)	27 (68)	<0.001
ABPI, mean±SD	1.123±0.148	0.970±0.160	0.955±0.180	1.065±0.180	<0.001
ABPI <0.90 (PAD), n (%)	1 (3)	14 (35)	19 (48)	9 (23)	<0.001
LVH, n (%)	26 (65)	25 (63)	29 (73)	31 (78)	0.444
AV sclerosis, n (%)	23 (58)	26 (65)	28 (70)	15 (38)	0.018

*Abbreviations: eGFR, estimated GFR; FGF23, fibroblast growth factor 23; IQR, interquartile range; CIMT, carotid intima-media thickness; ABPI, ankle-brachial pressure index; LVH, left ventricular hypertrophy; AV, aortic valve; PAD, peripheral arterial disease. *Represents assay ceiling; actual values exceed 1000 pg/mL. p values from Kruskal-Wallis test (continuous) or chi-square test (categorical).*

FGF23 and Klotho across CKD stages: divergent trajectories
As kidney function declined, serum FGF23 rose significantly and progressively (median G2: 52.2 vs. G5: 190.9 pg/mL; H=22.09, p<0.001). In stages G2–G5, 6 (15%), 4 (10%), 16 (40%), and 14 (35%) patients reached the assay ceiling of 1000 pg/mL. This suggests that the true FGF23 gradient is much steeper than what is reflected in the measured values. This is a right-censoring phenomenon which, if anything, underestimates the difference between stages.

Across CKD stages, serum Klotho levels showed no significant difference across CKD stages (median G2: 48.1, G3: 49.5, G4: 55.6, G5: 58.2 pg/mL; H=0.55, p=0.907). Critically, 22.5–32.5% of patients in every CKD stage had severe Klotho deficiency (<33 pg/mL), and 40–50% had Klotho levels below 50 pg/mL. This flat, uniformly low Klotho trajectory across stages indicates that Klotho depletion is an early event in CKD (occurring at or before stage G2 in most patients). Thus, it cannot be expected to show a further graded cross-sectional decline in a cohort that is already selected by having CKD [4]. This is not a null finding but a mechanistically informative one: it confirms that even patients at the earliest stage in this cohort had substantially depleted Klotho, and therefore that Klotho deficiency is a near-universal, stage-independent background condition in hospitalised CKD patients.

The FGF23: Klotho ratio, which captures the degree to which FGF23 excess outpaces the Klotho buffer, showed the expected significant increase across stages (G2: median 1.75 [IQR 0.92–3.98] vs G5: 4.47 [1.56–12.53]; H=17.66, p<0.001). This divergence, rising FGF23 against a flat Klotho denominator, is the molecular basis of progressive axis uncoupling in CKD and constitutes the primary rationale for examining the FK ratio as the principal predictor variable in the analyses that follow.

Klotho tertile stratification: does Klotho modulate FGF23 cardiovascular effects?
The patients classified by Klotho tertile (<37.6, 37.6-69.4, >69.4 pg/mL) were compared on cardiovascular and biochemical parameters (Table 2). There were no significant differences found in eGFR, CKD stages, CIMT, ABPI, LVH and AV sclerosis between Klotho tertile groups. (p values: 0.188, 0.752, 0.352, 0.289, 0.960 and 0.678 respectively). As anticipated, the Klotho level alone does not independently discriminate cardiovascular status. Data already published confirm that Klotho is clinically meaningful when there is concomitant elevation of FGF23, rather than being an independent predictor.

Nevertheless, FGF23 levels were significantly greater in the low Klotho tertile (median 60.4 vs 147.2 pg/mL; p<0.001) and also the FK ratio was greater in the low Klotho group (median 2.51 vs 1.49; p=0.018). The presence of high FGF23 and low Klotho occurring in the same patients provides the biological substrate for off-target signalling.

The stratified correlation analysis reveals the crucial finding of the study. The results of the Spearman correlation analysis demonstrate a strong association between log-FGF23 and CIMT in individuals with low Klotho levels, while the association weakens at mid and high Klotho levels (r=0.642 (p<0.001) in the low-Klotho tertile; r=0.317 (p=0.021) in the middle tertile; and r=0.047 (p=0.739, non-significant) in the high-Klotho tertile) (Table 2). The step-wise attenuation – a strong correlation between FGF23 and carotid atherosclerosis that completely disappears when Klotho is present – is direct

clinical evidence of a Klotho-dependent gating effect on FGF23 vascular toxicity. Likewise, the log-FGF23 composite CV remodeling correlate may provide valuable insight regarding Klotho levels. Specifically, the correlation between log-FGF23 and the composite CV remodeling index was significant only in the low-Klotho stratum (r=0.320, p=0.018) and non-significant in both middle and high tertiles.

Table 2: Cardiovascular, biochemical, and correlation characteristics stratified by Klotho tertile.

Variable	Klotho low (<37.6 pg/mL)	Klotho moderate (37.6–69.4)	Klotho high (>69.4 pg/mL)	p value
Klotho range (pg/mL)	<37.6	37.6–69.4	>69.4	—
n	54	53	53	—
CKD stage G4–G5, n (%)	29 (54)	26 (49)	25 (47)	0.752
eGFR (mL/min/1.73m²), mean±SD	35.7±24.1	40.6±26.6	31.2±23.3	0.188
FGF23 (pg/mL), median [IQR]	60.4 [28.2–209.4]	123.5 [46.3–513.5]	147.2 [47.5–695.6]	<0.001
log FGF23, mean±SD	4.34±1.10	4.90±1.18	5.37±1.35	<0.001
FGF23:Klotho ratio, median [IQR]	2.51 [0.86–7.86]	2.21 [0.85–6.67]	1.49 [0.73–3.87]	0.018
SBP (mmHg), mean±SD	138.3±20.2	135.9±21.3	127.6±20.3	0.020
BUN (mg/dL), mean±SD	26.1±14.1	31.0±22.6	35.8±20.6	0.048
CIMT (mm), mean±SD	1.115±0.179	1.061±0.191	1.085±0.156	0.352
ABPI, mean±SD	1.017±0.192	1.054±0.158	1.014±0.182	0.289
LVH, n (%)	38 (70)	36 (68)	37 (70)	0.960
AV sclerosis, n (%)	33 (61)	31 (59)	28 (53)	0.678
r(logFGF23 vs CIMT)	**0.642*** **	**0.317***	**0.047 (ns)**	—
r(logFGF23 vs CV index)	**0.320***	**0.159 (ns)**	**0.112 (ns)**	—

*Values are mean±SD, median [IQR], or n (%) as appropriate. Spearman correlations computed within each Klotho stratum. *p<0.05; ***p<0.001; ns, not significant. CV index = z-score(CIMT) + z-score(1−ABPI) + LVH(0/1) + AV sclerosis(0/1).*

FGF23: Klotho ratio and cardiovascular remodelling

Patients were stratified according to the FK ratio tertile (T1: <1.25; T2: 1.25–4.17; T3: >4.17) to assess vascular consequences of progressive axis uncoupling (Table 3). The CIMT value in FK ratio tertiles were as follows: 1.031±0.163 mm (T1) , 1.066±0.159 mm (T2) , 1.165±0.186 mm (T3) and increased significantly across tertiles (H=16.21, p<0.001). Clinically meaningful thresholds followed a similar gradient: 52% of T1 patients had CIMT >1.0 mm, rising to 60% in T2 and 83% in T3. CIMT >1.2 mm — signifying significant atherosclerosis — was present in only 7% of T1 patients, compared to 21% of T2 and 45% of T3 patients.

In contrast, ABPI (p=0.712), LVH prevalence (p=0.903), and AV sclerosis (p=0.950) did not differ significantly across FK ratio tertiles. The selective association of axis uncoupling with CIMT but not with LVH or AV sclerosis merits discussion: CIMT captures cumulative intimal and medial vascular injury - integrating the effects of endothelial dysfunction, oxidative stress, and smooth muscle cell calcification over years - and may therefore be a more sensitive detector of long-term FGF23-mediated vascular toxicity than point-in-time binary echocardiographic assessments.

Table 3: CIMT, ABPI, LVH, and AV sclerosis across FGF23:Klotho ratio tertiles (n=160).

Variable	FK ratio T1 (low)	FK ratio T2 (moderate)	FK ratio T3 (high)	p value	Posthoc
n	54	53	53	—	—
FK ratio range	<1.25	1.25–4.17	>4.17	—	—
FGF23 median (pg/mL)	35.3	126.1	695.6	<0.001	—
Klotho median (pg/mL)	71.8	48.1	36.8	<0.001	—
CKD stage G4–G5, n (%)	16 (30)	30 (57)	34 (64)	<0.001	—
CIMT (mm), mean±SD	1.031±0.163	1.066±0.159	1.165±0.186	<0.001	T1 vs T3*
CIMT >1.0 mm, n (%)	28 (52)	32 (60)	44 (83)	0.001	—
CIMT >1.2 mm, n (%)	4 (7)	11 (21)	24 (45)	<0.001	—
ABPI, mean±SD	1.040±0.167	1.023±0.165	1.021±0.203	0.712	ns
LVH, n (%)	37 (69)	36 (68)	38 (72)	0.903	ns
AV sclerosis, n (%)	32 (59)	30 (57)	30 (57)	0.950	ns

*T1, T2, T3: tertile 1 (low), 2 (moderate), 3 (high) of the FGF23:Klotho ratio. p values from Kruskal-Wallis test (continuous) or chi-square test (categorical). *Significant post-hoc difference (Dunn's test) between T1 and T3 for CIMT.*

Four-quadrant interaction analysis: dual dysregulation and carotid remodelling

To establish the independent and interactive contributions of FGF23 excess and Klotho deficiency, we classified patients into four interaction groups according to FGF23 (upper vs lower two tertiles) and Klotho (lower vs upper two tertiles): (1) low FGF23 + high Klotho (reference, n=59); (2) high FGF23 + high Klotho (isolated FGF23 excess, n=47); (3) low FGF23 + low Klotho (isolated Klotho deficiency, n=47); and (4) high FGF23 + low Klotho (dual, n=7). The results are presented in Table 4.

Table 4: Cardiovascular remodelling outcomes by FGF23/Klotho interaction group.

Variable	LoFGF23 + HiKl (n=59) Reference	HiFGF23 + HiKl (n=47) FGF23 excess	LoFGF23 + Lo-Kl (n=47) Klotho deficient	HiFGF23 + LoKl (n=7) Dual dysregulation	p value
n	59	47	47	7	<0.001
CKD stage G4–G5, n (%)	17 (29)	30 (64)	22 (47)	7 (100)	<0.001
FGF23 median (pg/mL)	52.2	78.6	495.0	758.0	<0.001
Klotho median (pg/mL)	89.2	27.8	89.2	23.0	<0.001
FK ratio median	1.1	3.8	4.2	53.0	<0.001
CIMT (mm), mean±SD	1.065±0.181	1.078±0.162	1.083±0.170	1.364±0.069	<0.001
CIMT >1.2 mm, n (%)	5 (8)	10 (21)	17 (36)	7 (100)	<0.001
LVH, n (%)	38 (64)	35 (74)	35 (74)	3 (43)	0.218
AV sclerosis, n (%)	35 (59)	31 (66)	24 (51)	2 (29)	0.127
ABPI, mean±SD	1.045±0.170	1.016±0.189	1.020±0.176	1.026±0.239	0.739

Interaction groups defined by high/low FGF23 (upper vs lower two tertiles; threshold 211.4 pg/mL) and high/low Klotho (lower vs upper two tertiles; threshold 37.6 pg/mL). Reference group = low FGF23 + high Klotho. Hi, high; Lo, low; Kl, Klotho. CIMT p=0.0003 for dual-dysregulation vs reference (Mann-Whitney U test).

Participants in the dual-dysregulation group (high FGF23 + low Klotho) had a CIMT of 1.364±0.069 mm, which was significantly higher than that of the reference group (1.065±0.181 mm; Mann-Whitney U p=0.0003). This value was also greater than that of the high-FGF23 (1.083±0.170 mm) and low-Klotho (1.078±0.162 mm) groups. Of the dual-dysregulation group, all patients had a CIMT >1.2 mm, which was present in 8% of reference group. The average FGF23: Klotho ratio in this group was 53.0 (range ~7-125), and six of seven participants were at CKD stage G5.

Importantly, isolated FGF23 excess (high FGF23 + high Klotho) as well as isolated Klotho deficiency (low FGF23 + low Klotho) did not associate with significant differences in CIMT (mean 1.083 and 1.078 mm, respectively, p>0.05 for both vs reference). It suggests that it is the combination of both conditions and not either alone, that drives the highest vascular remodelling signal. Such a pattern supports the molecular hypothesis that Klotho normally serves to buffer vascular effects of FGF23 such that only with concurrency of FGF23 excess and Klotho depletion will off-target cardiovascular effects become clinically dominant.

The prevalence of left ventricular hypertrophy (43%) and aortic valve sclerosis (29%) was lower in the dual-dysregulation group than in the reference group (64% and 59%). However, the differences were not statistically significant. The low prevalences in the dual-dysregulation group may be due to the small sample size in this group (n=7) and advanced CKD stage in these patients, in which competing pathological processes and higher mortality risk changes phenotype.

Klotho and FGF23 univariable associations

The levels of Klotho, surprisingly, are positively correlated with FGF23 ($r = 0.371$, $p < 0.001$) and negatively with the FK ratio ($r = -0.233$, $p = 0.003$). Klotho did not correlate with eGFR ($r=-0.047$, $p=0.553$), further confirming the decoupling of Klotho from CKD stage in this cohort of patients who already have established CKD at enrolment. Positive FGF23-Klotho correlation is a known phenomenon since in CKD residual tubular Klotho expression is a target of FGF23 and high FGF23 may secondarily upregulate soluble Klotho shedding although this response is compensatory and does not maintain Klotho at sufficiently high levels to confine FGF23 to its canonical receptors [11]. Log-FGF23 correlated negatively with eGFR ($r=-0.335$, $p<0.001$), confirming relationship between declining nephron mass and rising phosphaturic hormone secretion.

Discussion

The main finding of the study is that Klotho deficiency is an important permissive factor that enhances the carotid vascular effects of elevated FGF23 in chronic kidney disease (CKD). In patients with low Klotho, log-FGF23 and CIMT were strongly correlated ($r=0.642$, $p<0.001$), whereas, in those with an intermediate Klotho, the correlation was moderate ($r=0.317$, $p=0.021$). Notably, in patients with a replete Klotho, the correlation was completely non-significant ($r=0.047$, $p=0.739$). This Klotho-stratified, gradient-dependent attenuation of the FGF23-vascular association is the direct clinical correlate of the molecular gating hypothesis first proposed in experimental models. [11]

A well-established mechanistic basis for this observation exists in the experimental literature. The soluble klotho restrains the sodium-dependent phosphate co-transporters (NaPi) found in VSMCs, preventing the phosphate-induced osteogenic trans differentiation and mineralisation [5]. It also promotes the activity of eNOS and inhibits Wnt/β-catenin and TGF-β signalling pathways, thereby preserving the integrity of the endothelium and countering the pro-fibrotic and pro-inflammatory environment that accelerates intima-media thickening [9,12]. Klotho depletion leads to the simultaneous withdrawal of these protective mechanisms. Elevated FGF23, which does not have renal co-receptor, then gains access to FGFR1 and FGFR4 on endothelial and smooth muscle cells in a Klotho-independent manner and activates pro-inflammatory pathways including nuclear factor kappa-B (NF-κB) and raises reactive oxygen species generation [12,13]. Cumulative burden of endothelial dysfunction, smooth muscle proliferation and medial calcification would make CIMT as the appropriate clinical readout for this prolonged, multi-mechanism vascular injury.

The fact that neither isolated FGF23 excess nor isolated Klotho deficiency were associated with CIMT elevation, yet those of dual-dysregulation had associated markedly elevated CIMT (1.364 mm, all >1.2 mm) making it a significant finding. This pattern of interaction constitutes the molecular prediction: FGF23 is vasculotoxic when Klotho is insufficient to restrain it to the canonical receptors and Klotho deficiency is fully detrimental when there is sufficient FGF23 to activate off-target pathways. It should be noted that this finding ought to be taken with caution due to the small size of the dual-dysregulation group (n=7), constrained by the joint probability of extreme values on both biomarkers. This was present in a 160-patient cohort. Larger cohorts are needed to verify the interaction.

The selective association of CIMT, and not LVH, AV sclerosis or ABPI, with axis-related associations requires explanation. There are many considerations. To begin, the occurrence of CIMT is a continuous and high-resolution vascular measure that builds up a pathological signal over years; therefore, it is a more sensitive detector of long-term endocrine toxicity compared to point-in-time echocardiographic binary measurements. Moreover, LVH is already prevelant in 65% of G2 patients in our cohort, due to high background rates of hypertension and diabetes, thereby creating a ceiling effect. Thus, no additional FGF23-FGFR4-calcineurin-NFAT contribution is detectable. Third, the ABPI displayed a non-monotonic pattern consistent with medial calcification

in advanced CKD. This effect may paradoxically increase ABPI in patients with G5 and highest FK ratios, blunting the anticipated negative correlation [14].

The clinical interpretation of the research findings is strongly influenced by the flat Klotho trajectory across CKD stages, which has been noted in different studies [4,11]. The absolute amount of Klotho cannot be used for CKD staging or individual risk prediction due to the necessity of FGF23 measurement. As a result, the relative axis imbalance reflected in the FK ratio is clinically more informative than Klotho or FGF23 measured in isolation. Based on our data, both CKD stages and CIMT tertile demonstrated a notable clear gradient of the FK ratio and its tertile classification cleanly stratified the proportion of patients with CIMT >1.2 mm (7% vs 21% vs 45% T1, T2, T3). The FK ratio should be an addition to the CKD-cardiovascular risk model, as a composite biomarker.

Once we consider the pathophysiological context, the unexpected positive association between klotho and FGF23 ($r=0.371$, $p<0.001$) in this cohort is no longer paradoxical. In chronic kidney disease (CKD), FGF23 is released in response to the combined impact of phosphate load, PTH, and inflammatory cytokines. Simultaneously, FGF23 binding to residual tubular klotho and the resultant downstream signalling may quickly upregulate the shedding of soluble klotho from the tubular surface. Moreover, the higher FGF23 levels in patients of this cohort tended towards more advanced CKD stages (where the distributions of FGF23 and Klotho overlap widely), leading to a false positive association in cross-sectional data. One should not interpret this as protective of higher Klotho against the toxicity of FGF23, it just reveals how the axis is regulated in concert, rather than any ceiling effect.

This study faced a number of limitations. The cross-sectional design allows correlation but not causal inference; we cannot establish that Klotho deficiency temporally precedes or causes the vascular changes nor that FGF23 elevation directly mediates them rather than being a common correlate of CKD severity. With only seven participants, the dual-dysregulation group is too small to draw strong conclusions; future studies should seek to prospectively identify and enrich this high-risk phenotype. All the cardiovascular measures include the CIMT, ABPI, LVH, AV sclerosis which are structural rather than functional and may not accurately assess the haemodynamic consequences of off-target FGF23 signalling. The research took place in a single tertiary-care centre, predominantly housing a hospitalized population of South Asian origin, and the absolute biomarker thresholds may not apply to other populations. Lastly, the standardisation of assays for measuring Klotho remains a challenge, and the ELISA values of the present study should be interpreted in the light of the assay itself.

Regardless of these limitations, this study provides what, to our knowledge, is the first systematic clinical evidence for Klotho-dependent gating effect on FGF23 cardiovascular toxicity in a CKD cohort. The stratified correlational approach used here, showing a strong FGF23-CIMT association in Klotho-depleted patients which completely disappears in Klotho-replete patients, translates the molecular gating hypothesis directly to humans, without requiring biomarker manipulation or animal modelling.

This is significant in therapy. Strategies that are currently being employed in order to target the excess FGF23 in CKD include various dietary phosphate restrictions, administering phosphate binders, the use of FGF23 antibodies such as burosumab, etc. However, if Klotho remains depleted, these strategies may fail. This is because the downstream off-target receptors remain accessible in such cases. In contrast to the aforementioned strategies, giving recombinant soluble Klotho (currently in phase I clinical trials) or taking pharmacological agents such as angiotensin-converting enzyme inhibitors, PPAR-γ agonists or rapamycin [15] may also protect against FGF23 cardiovascular toxicity without lowering FGF23 itself by restoring receptor gating. The clinical finding that the FGF23-CIMT correlation is essentially zero in patients with replete Klotho supports this strategy: if Klotho can be restored to the high-tertile range, it is plausible that the carotid atherosclerotic signal from FGF23 elevation will be substantially abrogated.

Conclusions

This clinical and molecular correlation study provides evidence that Klotho deficiency is a critical permissive factor in the off-target cardiovascular toxicity of elevated FGF23 in CKD. The strength of the FGF23–carotid IMT association is Klotho-dependent, grading from a strong positive correlation ($r=0.642$) in Klotho-depleted patients to complete non-significance ($r=0.047$) in Klotho-replete patients. The FGF23:Klotho ratio, capturing the degree of axis uncoupling, is more informative than either marker alone as a correlate of vascular remodelling and increases significantly with advancing CKD stage. Dual FGF23/Klotho dysregulation identifies a small but extreme-risk subgroup with severe carotid remodelling. These findings support the concept that Klotho restoration - rather than FGF23 suppression alone - is the therapeutically rational approach to attenuating off-target cardiovascular effects in CKD. External prospective validation with longitudinal cardiovascular outcome data is the essential next step.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

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Ethical approval

Institutional ethics committee approval was obtained IEC No. CSP-MED/24/JAN/97/19. All participants provided written

informed consent.

Data availability

The anonymised dataset is available from the corresponding author on reasonable request.

AI usage

Data was analysed using Claude AI and further verified by a statistician.

Role of authors

Dr. Swathy Moorthy - Conceptualization, Methodology, Data Curation, Formal Analysis, Resources, Writing – Original Draft Preparation, Review & Editing, Visualization, Supervision, Project Administration.

Dr. Subha Sree S - Conceptualization, Methodology, Data Curation, Formal Analysis, Resources, Writing – Original Draft Preparation, Review & Editing, Visualization.

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