serum EPO levels positively correlated with circulating C-terminal (total) FGF23 concentrations (A), but not with circulating intact FGF23 concentrations (B).

Figure S4. Associations between ferritin and fibroblast growth factor 23 (FGF23) in the autosomal dominant polycystic kidney disease (ADPKD) bone biopsy cohort. In patients with ADPKD, serum ferritin levels tended to inversely correlate with circulating C-terminal (total) FGF23 concentrations (A), but not with circulating intact FGF23 concentrations (B) or with bone FGF23 levels (C).

Figure S5. Associations among iron-related parameters in autosomal dominant polycystic kidney disease (ADPKD) subjects. In patients with ADPKD, serum erythropoietin (EPO) levels positively correlated with erythroferrone (ERFE) (A), but ERFE did not correlate with hepcidin (B). Serum ferritin positively correlated with hepcidin (C).

Table S1. Multiple linear regression modeling, dependent variable: log-transformed C-terminal (total) fibroblast growth factor 23 (FGF23) (n = 78).

Table S2. Multiple linear regression modeling, dependent variable: log-transformed intact fibroblast growth factor 23 (FGF23) (n = 78).

Table S3. Multiple linear regression modeling, dependent variable: C-terminal (total) fibroblast growth factor (FGF) 23, excluding outlying point (n = 77).

Table S4. Multiple linear regression modeling, dependent variable: intact fibroblast growth factor (FGF)23, excluding outlying point (n = 77).

Supplementary References.

Supplementary Methods.

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Elevated Fibroblast Growth Factor 23 Levels Are Associated With Greater Diastolic Dysfunction in ESRD

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Life expectancy in individuals with chronic kidney disease (CKD) is unacceptably low, and cardiovascular disease remains the leading cause of death.[1] Above and beyond established cardiovascular disease risk factors that are highly prevalent in CKD patients, unique risk factors such as abnormal mineral metabolism are widely hypothesized to contribute to the pathogenesis of cardiovascular disease in CKD.

Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone produced mainly by osteocytes. As kidney function declines in CKD, FGF23 rises early and counteracts phosphate accumulation. Elevated FGF23 levels are independently associated with increased risk of cardiovascular disease and mortality in different populations, including among those with CKD.[2] In animal models and *in vitro*, FGF23 has a direct pathogenic effect, causing left ventricular (LV) hypertrophy by activating fibroblast growth factor receptor 4 on cardiac myocytes. In prior human observational studies, higher FGF23 has been associated with arrhythmias, and decreased LV systolic function.[3] Given animal data suggesting that FGF23 may induce LV hypertrophy, and human data demonstrating associations of elevated FGF23 with LV hypertrophy[4]—one potential pathologic driver of diastolic dysfunction—we hypothesized that higher FGF23 levels may be associated with diastolic dysfunction, a common complication of CKD.

METHODS

Please see Supplementary Material.

RESULTS

Baseline characteristics of the study cohort are shown in Table 1, stratified by quartiles of intact (i) FGF23. Seventy percent of the 47 participants were men, and 62% had preserved LV ejection fraction (EF). The mean (±SD) age was 61 (±20) years, mean phosphate was 5.0 (±1.4) mg/dl; mean LVEF was 51 (±13) %, mean left atrial volume was 58 (±27) ml, mean tricuspid velocity was 2.6 (±0.5) m/sec, and the mean E (transmitral early filling velocity)/A (transmitral late filling velocity) ratio was 1.4 (±0.5). A total of 94% (44 of 47) of patients had LV diastolic dysfunction, and 53% (25 of 47) had LV hypertrophy (LVH).

Median iFGF23 was elevated at 1135 (interquartile range: 361, 3195) pg/ml, which is comparable to values observed in end-stage kidney disease cohorts.[5] The strongest association was between higher levels of iFGF23 and grades of diastolic dysfunction ($r_s = 0.75$; $P < 0.001$), followed by serum phosphate and iFGF23 ($r_s = 0.51$; $P < 0.001$). In the univariate model, elevated levels of natural log–transformed iFGF23 were significantly associated with a higher grade of LV diastolic dysfunction ($R^2 = 0.51$; 95% confidence interval for slope, 1.7–3.4; $P < 0.001$). In a multivariate model, this relationship remained significant and was essentially unaltered after adjusting for age, phosphate, and LVEF ($R^2 = 0.5$; 95% confidence interval for slope, 1.01–1.5; $P < 0.001$; Figure 1).

DISCUSSION

In this study of 47 patients with end-stage kidney disease treated with hemodialysis, we found that higher levels of iFGF23 were associated with LV diastolic dysfunction. The association appeared to have a step-wise linear relationship with grades of severity of diastolic dysfunction, independently of LVEF. Although observational data reported here cannot prove causality, these data parallel observations made

Table 1. Baseline characteristics by quartiles of FGF23

Variable[a]	Full cohort	Quartile 1	Quartile 2	Quartile 3	Quartile 4
N	47	9	12	14	12
iFGF23 (pg/ml)		32–361	363–1074	1195–3195	3926–22,369
Age (yr)	61 (±20)	66 (±22)	57 (±18)	55 (±18)	68 (±21)
Weight (kg)	68 (±18)	61 (±16)	80 (±18)	66 (±19)	63 (±12)
Male (%)	70	67	75	64	75
EF ≥ 50%, n (%)	29 (62)	5 (56)	9 (75)	10 (71)	5 (42)
LV hypertrophy[b] n (%)	25 (53)	4 (44)	5 (42)	6 (43)	10 (83)
TR jet velocity (cm/s)	2.6 (±0.5)	2.5 (±0.7)	2.3 (±0.3)	2.7 (±0.3)	2.9 (±0.3)
E to A ratio[c]	1.4 (±0.5)	0.9 (±0.4)	1.1 (±0.6)	1.5 (±0.6)	2.1 (±0.4)
LA volume (ml)	58 (±27)	56 (±24)	55 (±25)	55 (±21)	65 (±37)
Hemoglobin (g/dl)	11.7 (±1.4)	11.4 (±1.0)	11.4 (±1.0)	12.2 (±1.5)	11.5 (±1.7)
Calcium (mg/dl)	8.6 (±0.7)	8.5 (±0.5)	8.6 (±0.4)	8.6 (±0.7)	8.6 (±0.9)
Phosphate (mg/dl)	5 (±1.4)	3.9 (±0.8)	4.7 (±1.0)	5.4 (±1.3)	5.8 (±1.7)

EF, ejection fraction; iFGF, intact fibroblast growth factor; LA, left atrium; LV, left ventricle; TR, tricuspid regurgitation
[a]Data are reported as mean (±SD) for continuous variables, and % for categorical variables.
[b]Left ventricular wall thickness >11 mm is defined as LV hypertrophy.
[c]E (transmitral early filling velocity) / A (transmitral late filling velocity) ratio.

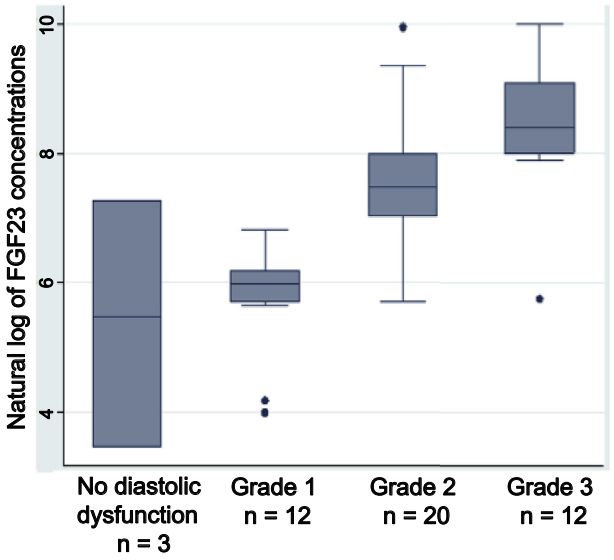

Figure 1. Box plots showing the relationship between the grading of diastolic dysfunction and the natural logarithm of intact fibroblast growth factor 23 (FGF23) in 47 hemodialysis patients. Elevated levels of natural log–transformed intact FGF23 were significantly associated with a higher grade of left ventricular diastolic dysfunction ($R^2 = 0.51$; 95% confidence interval for slope, 1.7–3.4; $P < 0.001$).

in vitro and in experimental animals demonstrating that FGF23 induces LVH. This suggests that a similar biology may be ongoing in hemodialysis patients, promoting diastolic dysfunction. Because diastolic dysfunction is strongly associated with mortality and morbidity, it is important to identify its potential drivers. Future studies are needed to determine if FGF23 lowering may lead to less diastolic dysfunction in hemodialysis patients.

Among its myriad effects, FGF23 mediates cardiac fibrosis through the activation of pro-fibrotic factors in *in vitro* models,[6] as well as in CKD populations,[7] thereby pointing to a plausible biological link to cardiac diastolic dysfunction. Our data expand on findings in pediatric nondialysis CKD patients where high FGF23 and low Klotho levels were strongly and longitudinally associated with LV diastolic function.[8] In contrast, in a retrospective study of subjects with LVEF >50% and relatively preserved renal function (mean estimated glomerular filtration rate of 52 ml/min per 1.73 m^2), FGF23 was not associated with diastolic dysfunction.[9] In comparison to those in the later study, our study population had much more severe kidney failure, and much higher FGF23 concentrations.

Our study also confirms that FGF23 correlates with hyperphosphatemia, a known risk factor for LVH and mortality in end-stage renal disease. LVH is known to be strongly associated with mortality and also associated with diastolic dysfunction by increasing LV filling pressure, leading to left atrial enlargement. Although others have shown an association between FGF23 and LVH, 47% of our patients did not have LVH. This latter finding suggests that other mechanisms independent of LVH may contribute to myocardial remodeling associated with impairment of diastolic function.

Our study has limitations. Our sample size is modest and applicable to adult end-stage renal disease patients supported by hemodialysis. We cannot demonstrate causality or temporality, but building on previous evidence that FGF23 causes cardiac fibrosis *in vitro* and in animal models, our findings support the hypothesis that FGF23 is a risk factor for LV diastolic dysfunction in end-stage renal disease patients. Repeated measurements of FGF23 and other covariates, such as blood pressure at the time of echocardiographic examination, duration between last dialysis session and echocardiogram, and other cardiovascular risk factors known to affect diastolic function, were not available. Given that FGF23 is not effectively removed by dialysis,[S1] we expect levels to be stable over time. Aside from CKD and hemodialysis status, the presence of comorbid conditions that could potentially confound the severity of diastolic function (diabetes, hypertension, prior myocardial infarction, dysrhythmias) was not known due to the retrospective nature of this study.

In conclusion, this study is the first to our knowledge to report an association between higher iFGF23 levels and severity of LV diastolic dysfunction in end-stage renal disease patients receiving hemodialysis. FGF23 has multiple adverse effects on the cardiovascular system, and understanding of the complex interplay of these effects in CKD patients is evolving. Future larger studies examining the relationship between FGF23 and the progression of diastolic dysfunction are warranted in patients with CKD, and ultimately, studies that target FGF23 lowering should be conducted to evaluate the effects on cardiac function.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

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Molecular Genetic Diagnosis of Omani Patients With Inherited Cystic Kidney Disease

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Chronic kidney disease (CKD) is defined as abnormalities in the structure or function of the kidney that are present for more than 3 months and have implications for health. Inherited kidney diseases are a major cause of CKD and often lead to progressive CKD resulting in end-stage renal disease (ESRD). Cystic kidney diseases are common inherited causes of ESRD in both children and adults, accounting for 6%–12% of cases.[S1,S2]

Inherited forms of cystic kidney have been associated with dysfunction of the primary cilia.[S3] These diseases are often termed renal ciliopathies and are part of a growing number of inherited diseases that include autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD),[S4] tuberous sclerosis complex (TSC),[S5] autosomal dominant tubulointerstitial kidney disease (ADTKD),[S6] nephronophthisis-related ciliopathies (NPHP-RC),[S7] Bardet-Biedl syndrome, Senior-Löken syndrome, Meckel Gruber syndrome, Joubert syndrome, and others.[S8]

ADPKD is the most common autosomal dominant inherited ciliopathy, accounting for 10% of all patients with ESRD requiring renal replacement therapy.[S2] It is characterized by bilateral renal cysts, leading to enlarged kidneys and kidney failure. Extrarenal manifestations such as liver cysts, intracranial aneurysms, and mitral valve prolapse are also frequently noted. Most cases of ADPKD are caused by mutations in *PKD1* (85%) and *PKD2* (15%), although recently mutations in *GANAB*[S9] and *DNAJB11*[S10] have been associated with similar phenotypes in genetically unresolved