Methods

Study Population

This study was approved by the local Institutional Review Board and all participants provided written informed consent. This cohort was previously described.[S2] Using a cross-sectional study design, we analyzed the association between intact FGF23 (iFGF23) and diastolic heart function in 47 prevalent adult hemodialysis (HD) patients. Inclusion criteria included end stage kidney disease supported by HD between 2009 and 2011 and clinical echocardiographic studies within 6 months of drawing iFGF23.

Primary Exposure and Outcome

We measured iFGF23 in duplicate using a second-generation assay (intact; Immutopics/Quidel) after a single thaw of frozen plasma samples that were collected at the beginning of the subjects' hemodialysis sessions and stored at –80°C. The mean intra-assay coefficient of variation was 2%, the mean inter-assay coefficient of variation was 3.5%, and the lower limit of detection was 1.5 pg/ml. The primary outcome was LV diastolic function, which was graded using multi-parametric Doppler echocardiographic analysis, as recommended by the 2005 American Society Echocardiography guidelines.[S3] The severity of diastolic dysfunction was graded based on mitral inflow velocities and mitral annular tissue velocities (cm/s). We defined left ventricular ejection fraction (LVEF) of ≥50% as "preserved" EF and LV hypertrophy (LVH) as LV wall thickness >1.1 cm.

Statistical Analysis

We examined continuous variables graphically and summarized baseline characteristics according to iFGF23 quartiles. Data are tabulated as mean ± standard deviation (SD) or median and IQR (interquartiles). Comparisons were made using ANOVA or ANOVA on ranks, as appropriate. Categorical variables were examined by frequency distribution, recorded as

proportions, and comparisons made using the chi-square test. We assessed the associations between iFGF23 and covariates (phosphate, age) using Spearman correlation coefficients. We used linear regression to evaluate the association between natural log-transformed iFGF23 levels and prevalent diastolic dysfunction. To investigate potential cardiac mediators of this association, we adjusted for serum phosphate, LVEF, and age. Statistical analyses were performed with SAS Version 9.4 (SAS Institute Inc., Cary, NC). Two-sided P <0.05 was considered statistically significant.

Supplementary References

S1. Miao LY, Zhu B, He XZ, et al. Effects of three blood purification methods on serum fibroblast growth factor-23 clearance in patients with hyperphosphatemia undergoing maintenance hemodialysis. *Experimental and therapeutic medicine.* Apr 2014;7(4):947-952. **5.**Zaritsky J, Young B, Wang HJ, et al. Hepcidin--a potential novel biomarker for iron status in chronic kidney disease. *Clinical journal of the American Society of Nephrology : CJASN.* Jun 2009;4(6):1051-1056.

S2. Zaritsky J, Young B, Wang HJ, et al. Hepcidin--a potential novel biomarker for iron status in chronic kidney disease. *Clinical journal of the American Society of Nephrology : CJASN.* Jun 2009;4(6):1051-1056.

S3. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography.* Dec 2005;18(12):1440-1463.