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Klotho and Fibroblast Growth Factor 23 Are Independent of Vitamin D, and Unlike Vitamin D, Are Not Associated With Graft- and Patient Survival After Kidney Transplantation

Inga Strand Thorsen, MD,[1,2] Inger Hjørdis Bleskestad, MD, PhD,[1] Anders Åsberg, PhD,[3,4,5] Grete Jonsson, PhD,[6] Øyvind Skadberg, MD,[6] Kristian Heldal, MD, PhD,[3,7] and Lasse Gunnar Gøransson, MD, PhD[1,2]

Background. Short-term survival after kidney transplantation is excellent but long-term survival remains suboptimal. The aim of the study was to explore the relationship between soluble α-Klotho (sKlotho) and intact fibroblast growth factor 23 (iFGF23) measured 8 wk and 1 y posttransplant with long-term graft- and patient survival in a cohort of kidney transplant recipients with deficient and nondeficient vitamin D (25[OH]D) levels. **Methods.** Vitamin D, sKlotho, and iFGF23 were measured 8 wk and 1 y posttransplant in 132 recipients transplanted between November 2012 and October 2013. **Results.** Of the 132 kidney transplant recipients, 49 had deficient vitamin D levels (<30 nmol/L) and 83 had nondeficient vitamin D levels (≥30 nmol/L) at 8 wk posttransplant. The mean age was 51 y and the median follow-up was 7.4 y. At 1 y posttransplant, vitamin D increased significantly. There were no significant differences in sKlotho or iFGF23 levels between the 2 vitamin D groups neither at 8 wk nor 1 y. sKlotho increased significantly and iFGF23 decreased significantly in the whole cohort. During the follow-up, there were 36 graft losses (27%) and 27 deaths (20%). Ninety-four percent of the transplant recipients with nondeficient vitamin D levels were alive with a well-functioning graft after 5 y using Kaplan-Meier survival estimates, compared with 84% of the patients with deficient vitamin D levels ($P = 0.014$). Klotho and FGF23 levels did not influence graft- and patient survival. **Conclusions.** In this nationwide cohort of kidney transplant recipients, long-term graft- and patient survival were significantly better in patients with vitamin D ≥30 nmol/L 8 wk posttransplant compared with those with vitamin D <30 nmol/L. sKlotho levels increased and iFGF23 levels decreased from 8 wk to 1 y posttransplant. Klotho and FGF23 levels were not associated with graft- and patient survival.

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Kidney transplantation is the preferred renal replacement therapy for most patients with end-stage renal disease (ESRD).[1,2] Observational studies show that a successful kidney transplantation is associated with a substantial reduction in the risk of mortality and cardiovascular events, as well as a relevant improvement in quality of life, compared with patients remaining in dialysis.[1,2] Short-term results after kidney transplantation have become excellent during recent decades, but long-term prognosis has not improved substantially

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[1] Department of Internal Medicine, Stavanger University Hospital, Stavanger, Norway.

[2] Department of Clinical Medicine, University of Bergen, Bergen, Norway.

[3] Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway.

[4] Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway.

[5] Norwegian Renal Registry, Oslo, Norway.

[6] Department of Medical Biochemistry, Stavanger University Hospital, Stavanger, Norway.

[7] Institute of Health and Society, University of Oslo, Oslo, Norway.

I.S.T. participated in the design of the study, data analysis, and writing and reviewing the article. I.H.B. participated in critically reviewing the article. A.Å. participated in the design of the study, supplying data from the Norwegian Renal Registry, data analysis, and critically reviewing the article. G.J. and Ø.S. participated in the analysis of Klotho and FGF23 and critically reviewing the article. K.H. participated in writing and critically reviewing the article. L.G.G. participated in making the study design, data analysis, and writing and critically reviewing the article.

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Correspondence: Inga Strand Thorsen, MD, Department of Internal Medicine, Stavanger University Hospital, Gerd Ragna Bloch Thorsens gate 8, 4011 Stavanger, Norway. (ings@sus.no).

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since the 1990s.[3] The mortality risk in transplant recipients is still very high compared with the general population, and 5-y survival after transplantation is actually comparable with the prognosis associated with many invasive malignancies.[4] Cardiovascular disease (CVD) is the number 1 cause of death, followed by malignant diseases and infections.[5]

Chronic kidney disease (CKD)-mineral bone disorder is a term that describes a broad clinical syndrome that develops as a systemic disorder of mineral and bone because of CKD. The syndrome manifests in bone and mineral abnormalities, histological bone disease, calcification of vasculature and soft tissues, and increased cardiovascular morbidity and mortality.[6]

Vitamin D is a steroid hormone with many effects in a human body. One of the most important roles of vitamin D is to maintain skeletal calcium homeostasis. 25 hydroxy-vitamin D (25[OH]D) is the form of vitamin D measured in serum and reflects body stores.[7] Increased parathyroid hormone (PTH) secretion induces calcitriol formation. Calcitriol, on the other hand, inhibits the synthesis and secretion of PTH, providing negative feedback regulation of calcitriol.[8] The levels of vitamin D are hypothesized to affect the immune system and may have protective effects against cancer,[9] CVD,[10] infection,[11] and mortality.[9] Vitamin D deficiency can result in reduced bone density, osteoporosis, and increased risk of fractures.[12]

Arterial stiffness can be estimated by measuring pulse wave velocity (PWV), which is the velocity at which the blood pressure (BP) pulse propagates through the circulatory system. PWV is the gold standard for a noninvasive evaluation of arterial stiffness.[13] Increased PWV predicts future cardiovascular events and all-cause mortality independently of conventional risk factors.[14] Calcification of central arterial vessels contributes to increased PWV. PWV is recognized as an indicator of target organ damage and a useful additional test in the investigation of hypertension.[14]

Fibroblast growth factor 23 (FGF23) is a circulating phosphaturic hormone secreted mainly from osteocytes in bone, acting on the kidneys and parathyroid glands to regulate phosphate metabolism and vitamin D metabolism.[15] Circulating FGF23 increases early in the development of CKD to increase phosphate excretion.[15] At a certain point, this mechanism is overloaded, and in dialysis patients, FGF23 levels are increased up to 1000 times the normal range.[16] FGF23 is synthesized as an inactive form, and it is activated when a signal peptide is cleaved off and the active form, the intact FGF23 (iFGF23) peptide, is formed.[17] FGF23 inhibits 1-α-hydroxylase in the kidney, which leads to reduced vitamin D levels.[18] Elevated FGF23 levels are also associated with CVD,[19] mortality,[19] and reduced graft function posttransplant.[20] FGF23 can be measured with different immunoassays, where some measure iFGF23, and others measure C-terminal FGF23.[21] In this study, we measured iFGF23.

Klotho was discovered in 1997 as an antiaging membrane protein in a mouse model of human aging. In animal studies, Klotho has kidney protective characteristics and participates in the calcium–phosphate homeostasis, in addition to antiaging and cardiovascular protective properties.[22] The Klotho gene encodes a transmembrane protein called α-Klotho that contains a short intracellular domain and an extracellular domain. Klotho protein exists in several forms, including the membrane-bound full-length form and a soluble circulating form (sKlotho).[23] The membrane-bound Klotho acts as a coreceptor for FGF23, and the tissue-specific Klotho expression influences the organ-specific effects of FGF23.[22,23] sKlotho is present in the blood, urine, and cerebrospinal fluid where it performs a multitude of functions, but the details are not fully understood.[23] sKlotho was measured in the present study. sKlotho is stimulated by vitamin D receptor activators[24] and by inhibition of the renin–angiotensin–aldosterone system.[25] sKlotho levels are downregulated in the presence of albuminuria, inflammation, and acute kidney injury.[26] sKlotho levels are reduced when kidney function declines,[27] and low levels of sKlotho are associated with increased risk of cardiovascular events and mortality in patients with CKD and ESRD.[28]

In a previous study,[29] we found better long-term graft- and patient survival in 762 first-time kidney transplant recipients with vitamin D sufficiency compared with patients with vitamin D insufficiency and deficiency. This study aimed to explore the relationship between sKlotho and iFGF23 measured 8 wk and 1 y posttransplant with long-term graft- and patient survival in 2 groups of kidney transplant recipients with deficient and nondeficient vitamin D levels 8 wk posttransplant.

PATIENTS AND METHODS

This prospective, observational cohort study included 132 kidney transplant recipients transplanted between November 19, 2012, and October 31, 2013. In Norway, solid organ transplantation was centralized in 1 center, Oslo University Hospital, Rikshospitalet (OUS-RH). A total of 236 patients received a single kidney transplant in the same period, 170 from a deceased donor and 66 from a living donor. Thirty-four of the 236 were retransplantations. Eight wk posttransplant, all patients were subjected to an in-depth investigation at the laboratory for Renal Physiology at OUS-RH, where vitamin D levels were analyzed and blood samples biobanked. We randomly selected 132 transplant recipients based on vitamin D levels only without taking any other information into consideration. Patients with a functioning kidney graft 1 y posttransplant were invited to OUS-RH for clinical examination and laboratory follow-up. One hundred twenty-eight of the 132 patients included (97%) attended the 1 y follow-up.

The standard immunosuppression regimen included basiliximab and methylprednisolone induction. The maintenance immunosuppression consisted of a calcineurin inhibitor (tacrolimus or cyclosporine) in combination with mycophenolate and prednisolone.[30] National guidelines regulate the immunosuppressive protocol, and the local nephrologists do the follow-up after the first 8 wk posttransplant.

In the routine in-depth investigation 8 wk and 1 y posttransplant, data on vital signs, BP, and blood samples for clinical chemistry were obtained. All patients were examined with aortic (carotid-femoral) PWV using SphygmoCore. The blood samples were collected after an overnight of fasting (food, drugs, and other beverages than water), and routine laboratory samples, including 25(OH) D, were analyzed in fresh samples at the Department of Medical Biochemistry at the transplant center. Blood samples were also biobanked at –70 °C in the Diagnostic and Treatment biobank "Nyrefysiologisk laboratorium" (Biobank no. 266-2005-142234) within 1 h. Plasma samples for analysis of sKlotho and iFGF23 were obtained from the biobank and measured en bloc at Stavanger University Hospital. The samples were only thawed once, and the biomarker results were not reported to the treating physicians.

Long-term outcomes from the time of transplantation until graft failure, death, or end of study on March 1, 2022, were retrieved from the Norwegian Renal Registry (NRR), where annual data are collected on the entire Norwegian transplant population. The reporting to the NRR is closely monitored, and the coverage for individuals and annual data are >99.9% and 96% to 98%, respectively.

The study was approved by the Regional Medical and Health Research Committee in South-East Norway (2014/455). Written and informed consent was obtained from all patients before any data or biological material were included in the NRR and the biobank at the transplant center.

Serum creatinine values were calibrated using the isotope dilution mass spectrometry method (reference range: 45–90 μmol/L [female]; 60–105 μmol/L [male]), and estimated glomerular filtration rate (eGFR) was calculated using the original CKD Epidemiology Collaboration equation.[31]

Glycosylated hemoglobin (HbA1c) results were given in % and converted to mmol/mol using the formula: International Federation of Clinical Chemistry and Laboratory Medicine HbA1c mmol/mol = (10.931 × National Glycohemoglobin Standardization Program − HbA1c) − 23.524.[32]

In fresh serum samples, 25(OH)D, including both 25-OH vitamin D_2 and 25-OH vitamin D_3, was measured by reversed-phase liquid chromatography coupled with tandem mass spectrometry detection. We used the National Institute of Health definition for vitamin D levels, with vitamin D deficiency defined as serum 25(OH)D concentrations <30 nmol/L (<12 ng/mL),[33] and we pooled insufficiency 30 to 50 nmol/L (12–20 ng/mL) and sufficiency >50 nmol/L (>20 ng/mL) as nondeficient vitamin D levels (≥30 nmol/L) to increase the clinical relevance and the statistical power.

Both iFGF23 and sKlotho were measured in ethylenediaminetetraacetic acid (EDTA) plasma. iFGF23 was measured with the commercially available human iFGF23 enzyme-linked-immunosorbent serologic assay (ELISA) kits from Immutopics International (San Clemente, CA), and sKlotho was measured with the commercially available human sKlotho ELISA kit from Immuno-Biological Laboratories (IBL, GmbH, Japan). Freshly thawed samples were analyzed in duplicates, and the mean value was reported. Coefficients of variation between duplicates were <17% for sKlotho and <16% for iFGF23. The intra-assay and interassay variability of the methods was <11% and <12% for iFGF23, respectively, and <13% and <12% for sKlotho, respectively (n = 3–6). iFGF23 in EDTA plasma in 8 healthy adults measured in the same local laboratory was with a mean of 44 ± 19 pg/mL.[34] sKlotho, measured by IBL ELISA kit in serum from 142 healthy participants, was reported to range from 239 to 1266 pg/mL, with a mean of 562 ± 146 pg/mL, in the original publication on the method.[35] In a group of 35 healthy controls measured at Stavanger University Hospital, serum sKlotho levels ranged from 458 to 1222 pg/mmol, with a median of 525 pg/mmol.[36] We did not analyze sKlotho in plasma from healthy controls, but a review article on sKlotho and healthy middle-aged adults reported plasma sKlotho levels in 14 studies averaged between 600 and 700 pg/mmol.[37] Another article analyzed sKlotho in serum and plasma using 3 different ELISA kits, one of them being the IBL kit. They found that measurements in serum and EDTA plasma were in agreement.[38]

Statistical Analysis

Normally distributed data are presented as mean ± SD and skewed data as median and range.

To compare the different variable distribution between vitamin D groups, analysis of variance was used for numeric variables and the chi-square test for categorical variables. Analysis of variance and paired samples *t* tests were used to compare variables measured at 8 wk posttransplant and at the end of 2020.

Survival analysis was performed using Kaplan-Meier estimate and multivariable Cox proportional hazard analysis, including iFGF23, sKlotho, vitamin D, delayed graft function (DGF), age, body mass index, eGFR, HbA1c, and hemoglobin measured at 8 wk as independent variables. The selection of variables was based on clinical knowledge and previous publications, and the number of variables had to be limited because of the low number of events. The proportionality assumption was checked using a log-minus-log plot. Two different Cox regression models were performed using uncensored graft loss (death and death-censored graft loss) and death as dependent variables.

To compare variables measured at 8 wk and 1 y posttransplant, a 2-sided *P* value was estimated using a paired sample *t* test for normally distributed variables and Wilcoxon signed-rank test for nonnormally distributed variables.

Statistical analysis was performed using IBM SPSS Statistics version 26. A *P* value of ≤0.05 was considered statistically significant.

RESULTS

iFGF23, sKlotho, and Vitamin D Status 8 Wk Posttransplant

Of the 132 selected kidney transplant recipients, 49 (37%) had 25(OH)D levels <30 nmol/L and 83 (63%) had 25(OH)D levels ≥30 nmol/L. The mean age was 51.1 y ± 12.6, and 58% were male. There were no significant differences in iFGF23 levels ($P = 0.17$) or sKlotho levels ($P = 0.37$) between the vitamin D groups at 8 wk (Table 1). The median follow-up time was 7.4 (1.2–8.1) y.

iFGF23, sKlotho, and Vitamin D Status 1 Y Posttransplant

In total, 128 of 132 patients attended the 1 y follow-up at the transplant center. In 4 of the 128 attendees, vitamin D measurement was missing, and all other measurements were registered. In the cohort as a whole, vitamin D level increased from a mean of 43 ± 20 nmol/L 8 wk posttransplant to 60 ± 26 nmol/L ($P < 0.001$) 1 y posttransplant. Thirteen patients (11%) had 25(OH)D levels <30 nmol/L, and 111 patients (87%) had 25(OH)D levels ≥30 nmol/L 1 y posttransplant. In the whole group, iFGF23 levels decreased from a median of 110 (15–1990) pg/mL to 89 (32–727) pg/mL ($P < 0.001$) from 8 wk to 1 y posttransplant. In the same period, sKlotho levels increased from a mean of 417 ± 129 pg/mL to 637 ± 207 pg/mL ($P < 0.001$), eGFR increased from a mean of 62 ± 19 mL/min/1.73 m^2 to 73 ± 25 mL/min/1.73 m^2 ($P < 0.001$), and intact PTH levels decreased from a median of 10.5 (2.3–132.2) μmol/L to 8.9 (2.3–72.5) μmol/L ($P < 0.001$; Figure 1; Table 2).

Graft Survival

During the follow-up period, a total of 36 grafts were lost, and 27 (75%) of these were because of death with a

TABLE 1.

Baseline characteristics 8 wk posttransplant, for all patients as well as divided on the basis of baseline plasma vitamin D levels (8 wk posttransplant).

Variables	All patients (n = 132)	Vitamin D <30 nmol/L (n = 49)	Vitamin D ≥30 nmol/L (n = 83)	*P*
Age, y	51.1 ± 12.6	50.7 ± 12.1	51.3 ± 12.9	0.901
Sex m/f (% male)	77/55 (58%)	30/19 (61%)	47/63 (57%)	0.605
BMI (kg/m^2)	25.1 ± 4.2	25.0 ± 4.2	25.1 ± 4.2	0.789
D in dialysis	239 (0–3871)	303 (0–3871)	188 (0–11 549)	0.019[a]
Living donor, n (%)	33 (25%)	10 (20%)	23 (28%)	0.349
First Tx, n (%)	110 (83%)	39 (80%)	71 (86%)	0.309
Preemptive tx, n (%)	43 (33%)	12 (24%)	31 (37%)	0.128
DGF, n(%)	16 (12%)	11 (22%)	5 (6%)	0.005[a]
Vitamin D, nmol/L	42.6 ± 19.9	22.2 ± 5.6	54.6 ± 15.1	<0.001[a]
sKlotho, pg/mL	417 ± 129	404 ± 115	424 ± 138	0.369
iFGF23, pg/mL	110 (31–1990)	109 (49–1793)	111 (31–1990)	0.171
eGFR, mL/min/1.73 m^2	62 ± 19	61 ± 22	62 ± 17	0.808
Creatinine, μmol/L	116 ± 40	121 ± 48	113 ± 34	0.299
Hemoglobin, g/dL	12.0 ± 1.3	11.9 ± 1.2	12.0 ± 1.4	0.651
Calcium, mmol/L	2.40 ± 0.13	2.41 ± 0.14	2.39 ± 0.12	0.722
iPTH, μmol/L	10.5 (2.3–132.3)	11.3 (2.5–132.3)	9.9 (2.3–48.0)	0.032[a]
Phosphate, mmol/L	0.93 ± 0.28	0.95 ± 0.30	0.86 ± 0.27	0.594
Albumin, g/L	41.6 ± 2.9	41.0 ± 3.3	41.8 ± 2.6	0.096
HbA1c, mmol/mol	40.7 ± 11.3	42.0 ± 13.2	39.9 ± 9.9	0.311

Values are presented as median (range) for dialysis duration, iFGF23, and iPTH and as mean ± SD for other normally distributed variables. ANOVA was used for numeric variables and the chi-square test for categorical variables.

[a]Significant difference between the groups ($P \leq 0.05$).

ANOVA, analysis of variance; BMI, body mass index; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; iFGF23, intact fibroblast growth factor 23; iPTH, intact parathyroid hormone; sKlotho, soluble α-Klotho; Tx, transplantation.

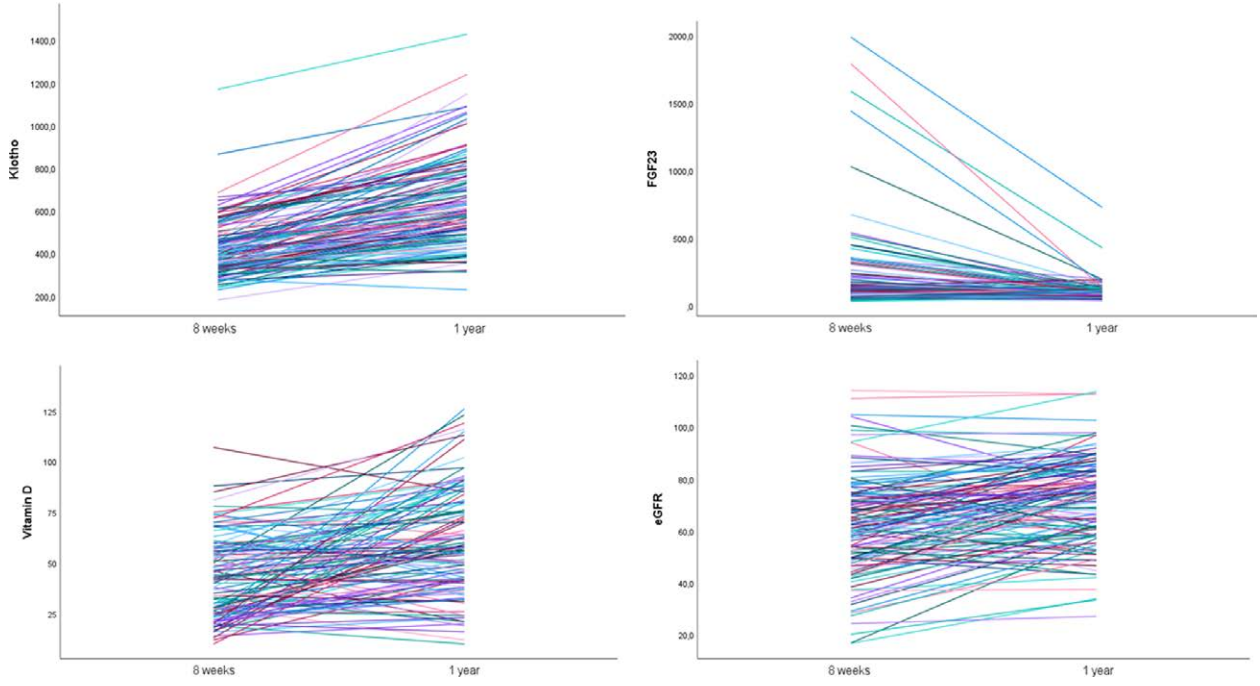


FIGURE 1. Spaghetti plots illustrating changes in Klotho, FGF23, vitamin D, and eGFR from 8 wk to 1 y posttransplant. eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23.

functioning graft. Overall, graft survival was significantly different between the 2 vitamin D groups. Twenty patients (41%) with vitamin D deficiency (<30 nmol/L) at 8 wk posttransplant suffered graft loss or death compared with 16 patients (19%) in the nondeficient vitamin D group (≥30 nmol/L; ($P = 0.007$; Table 3). There were no significant differences in age ($P = 0.901$), PWV ($P = 0.521$), systolic/diastolic BP ($P = 0.117/P = 0.328$), or cholesterol levels ($P = 0.571$) between the patients in the 2 groups of vitamin D levels.

The proportion of preemptive transplantations and living donors was higher in the group of patients with nondeficient vitamin D levels (≥30 nmol/L), and the patients with deficient vitamin D levels (<30 nmol/L) had longer time in dialysis before transplantation and more DGF posttransplant (Table 1).

TABLE 2.

8 wk and 1 y data.

Variables	8 wk (n = 132)	1 y (n = 128)	*P*
Age, y	51.1 ± 12.6	52.5 ± 12.3	–
Vitamin D, nmol/L	42.6 ± 19.9	59.8 ± 26.0	<0.001
sKlotho, pg/mL	417 ± 129	637 ± 207	<0.001
iFGF23, pg/mL	110 (31–1990)	89 (32–727)	<0.001
eGFR, mL/min/1.73 m^2	62 ± 19	73 ± 25	<0.001
Creatinine, μmol/L	116 ± 40	110 ± 33	0.022
Calcium, mmol/L	2.40 ± 0.13	2.43 ± 0.19	0.093
iPTH, μmol/L	10.5 (2.3–132.3)	8.9 (2.3–72.5)	<0.001
HbA1c, %	40.7 ± 11.3	45.2 ± 16.0	<0.001
BMI	25.1 ± 4.2	25.8 ± 4.3	<0.001

Median (range) for iFGF23 and iPTH and mean ± SD for other normally distributed variables.
A 2-sided *P* value is estimated using paired sample *t* test in normally distributed variables and Wilcoxon signed rank test in nonnormally distributed variables.
BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; iFGF23, intact fibroblast growth factor 23; iPTH, intact parathyroid hormone; sKlotho, soluble α-Klotho.

TABLE 3.

Graft survival and overall survival.

	All patients (n = 132)	Vitamin D <30 nmol/L (n = 49)	Vitamin D ≥30 nmol/L (n = 83)	*P*
Death n (%)	28 (21%)	16 (33%)	12 (14%)	0.013[a]
Death-censored graft loss n (%)	11 (8%)	6 (12%)	5 (6%)	0.212
Uncensored graft loss n (%)	36 (27%)	20 (41%)	16 (19%)	0.007[a]

ANOVA was used to explore the differences between the 2 groups.
[a]Significant difference between the groups ($P \leq 0.05$).
ANOVA, analysis of variance.

Crude Kaplan-Meier–estimated 5-y uncensored graft survival was 84% ± 5% (SE) in the patients with deficient vitamin D levels, compared with 94% ± 3% in the patients with nondeficient vitamin D levels ($P = 0.014$). After a 7-y follow-up, the uncensored graft survival was 74% ± 6% and 84% ± 4%, respectively ($P = 0.007$; Figure 2).

In the Cox regression models with uncensored graft loss as the dependent variable, high age ($P = 0.04$), high HbA1c ($P = 0.03$), low hemoglobin ($P = 0.03$), and low vitamin D ($P = 0.04$) were associated with uncensored graft loss. In the Cox regression model, with patient survival as the dependent variable, high age ($P < 0.001$), high HbA1c ($P = 0.01$), and low vitamin D ($P = 0.03$) were associated with death. sKlotho, iFGF23, DGF, and eGFR at 8 wk were included as variables in both models but had no impact on the dependent variables. Hemoglobin did not influence the model with death as a dependent variable (Table 4).

In the adjusted model, the patients with vitamin D levels ≥30 nmol/L had a hazard ratio for uncensored graft loss of 0.46 (0.23–0.95) compared with patients with vitamin D levels <30 nmol/L. Using death as the main endpoint, the patients with vitamin D levels ≥30 nmol/L had a risk of death of 0.40 (0.17–0.92), compared with patients with vitamin D levels <30 nmol/L (Table 5).

At 8 wk, PWV had a median of 9.1 (5.3–22.8) m/s. In the patients who died during follow-up, PWV was higher, 11.4 (5.3–16.6) m/s, compared with 8.8 (5.6–22.8) m/s in the survivors ($P = 0.08$). At 1 y, PWV was unchanged with a median of 9.2 (5.9–19) m/s.

7-y Patient Follow-up

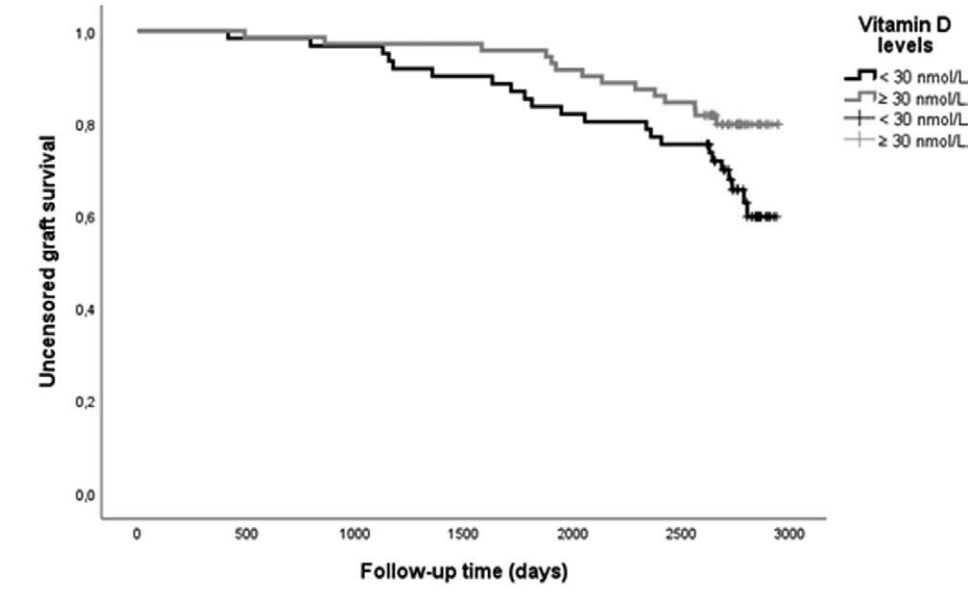
Ninety-six of 132 patients (73%) still had a functioning graft 7 y posttransplant, 29 of 49 patients (59%) with deficient vitamin D, and 67 of 83 patients (81%) with nondeficient vitamin D levels posttransplant ($P = 0.007$). The mean eGFR was 64 mL/min/1.73 m^2, with no significant difference between the 2 vitamin D groups. Intact PTH remained elevated above the normal reference range in both groups, with a higher mean level in the whole group of patients 7 y compared with 8 wk posttransplant, median (range) of 10.5 μmol/L (2.3–132.3 μmol/L) at 8 wk versus 11.0 μmol/L (2.7–44.7 μmol/L) after 7 y.

DISCUSSION

The main finding in the present study was that patients with vitamin D deficiency (<30 nmol/L) 8 wk posttransplant had reduced uncensored graft survival and reduced patient survival compared with the group that had vitamin D levels ≥30 nmol/L 8 wk posttransplant. sKlotho and iFGF23 at 8 wk showed no significant association with survival. High age, high HbA1c, low hemoglobin, and low vitamin D levels were associated with reduced graft survival, and high age, high HbA1c, and low vitamin D levels were associated with reduced patient survival.

Overall, the patients with functioning kidney grafts at the end of follow-up had higher eGFR at both 8 wk and 1 y posttransplant than the patients who died or lost their kidney. The most plausible explanation for the finding of higher eGFR 1 y after transplantation is the toxicity because of mandatory co-trimoxazole treatment the first 6 mo after transplantation. Somewhat higher calcineurin inhibitor levels in the early posttransplant phase may also contribute; at 8 wk, tacrolimus levels were 6.2 ± 1.7 μg/L compared with 5.9 ± 1.6 μg/L at 1 y after transplantation ($P = 0.043$). The "survivors" were almost 10 y younger at the time of transplantation.

The biochemical parameters of mineral metabolism often remain abnormal in transplant recipients for y after



Number at risk:

	0	500	1000	1500	2000	2500 (days)
<30 nmol/L	49	48	47	43	40	36
≥30 nmol/L	83	82	81	81	75	70

(vitamin D levels/ days of follow-up)

FIGURE 2. Kaplan-Meier plot showing uncensored graft survival in patients with deficient and nondeficient vitamin D levels.

TABLE 4.

HRs with 95% CIs and *P* values in the Cox regression models of graft- and patient survival, respectively.

	Uncensored graft loss		Death	
Variables	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
sKlotho	1.000 (0.997-1.003)	0.847	0.999 (0.995-1.003)	0.506
iFGF 23	1.000 (0.999-1.001)	0.989	1.001 (1.000-1.002)	0.227
eGFR	1.005 (0.999-1.001)	0.620	1.008 (0.985-1.031)	0.499
HbA1c	1.029 (1.003-1.055)	0.028	1.039 (1.008-1.071)	0.013
Vitamin D	0.464 (0.228-0.947)	0.035	0.379 (0.171-0.921)	0.031
Hb	0.699 (0.505-0.967)	0.030	0.977 (0.648-1.472)	0.910
Age	1.036 (1.002-1.070)	0.037	1.082 (1.040-1.136)	<0.001
DGF	1.377 (0.579-3.276)	0.470	1.451 (0.542-3.890)	0.506

Klotho, FGF23, eGFR, HbA1c, Hb, age, and d in dialysis are continuous variables; DGF and vitamin D are categorical variables with no DGF; and vitamin D deficiency are reference categories.
CI, confidence interval; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycosylated Hb; HR, hazard ratio; iFGF23, intact fibroblast growth factor 23; sKlotho, soluble α-Klotho.

transplantation, with persistent hyperparathyroidism, hypophosphatemia, and hypercalcemia.[39] One study including 52 kidney transplant recipients found that FGF23 levels were elevated before transplantation but decreased to normal levels within 3 mo posttransplant as eGFR normalized.[40] Another study reported elevated FGF23 levels 3 mo posttransplant,[41] and others have shown persistently elevated FGF23 levels in transplant recipients 1 y posttransplant.[42] Even in kidney transplant recipients with a well-functioning graft >10 y post-transplant, the levels of FGF23 were found to be increased compared with normal controls.[43] Elevated FGF23 posttransplant is associated with lower posttransplant eGFR,[20] and increased risk of graft loss,[44] cardiovascular,[19] and all-cause mortality in long-term kidney transplant recipients.[44]

Serum Klotho decreased in the initial postoperative phase after transplantation but increased rapidly after successful kidney transplantation, with the highest levels at 12 mo post-transplant.[42] One study showed that Klotho levels 7 d and 1 mo posttransplant were similar to Klotho levels before transplantation, but levels started to rise approximately 4 mo

TABLE 5.

HRs with 95% CIs in the unadjusted and the adjusted Cox regression models.

Model	Uncensored graft loss		Death	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Unadjusted				
Vitamin D <30 nmol/L	Reference		Reference	
Vitamin D ≥30 nmol/L	0.45 (0.23-0.86)	0.02	0.42 (0.2-0.89)	0.02
Adjusted[a]				
Vitamin D <30 nmol/L	Reference		Reference	
Vitamin D ≥30 nmol/L	0.46 (0.23-0.95)	0.04	0.40 (0.17-0.92)	0.03

[a]Adjusted for the following variables: age, eGFR, sKlotho, iFGF23, DGF, Hb, and HbA1c. All adjustment variables were set to their empirical means when computing the adjusted survival curves. Vitamin D as a categorical variable with vitamin D deficiency (<30 nmol/L as reference category).

CI, confidence interval; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycosylated Hb; HR, hazard ratio; iFGF23, intact fibroblast growth factor 23; sKlotho, soluble α-Klotho.

posttransplant.[45] Another study on long-term surviving kidney transplant recipients (>10 y after transplantation) showed a nonsignificant decline in Klotho levels[43]; this might be as expected from 10 y of aging.

In our study, the median iFGF23 levels declined but were still higher than in normal controls 1 y posttransplant,[34] suggesting a significant difference between kidney transplant recipients and healthy individuals. The mean sKlotho levels increased but were still lower than in normal controls at 1 y.[36] One y posttransplant, the sKlotho levels trend toward healthy controls, most likely as a result of increased eGFR.

Patients with CKD and kidney transplant recipients have the same traditional risk factors for CVD as the general population and also to disturbed mineral metabolism, including elevated levels of FGF23 and decreased levels of Klotho.[46] Elevated FGF23 levels and reduced Klotho levels are both associated with left ventricular hypertrophy and CKD progression.[47] Klotho reduces hypertension and attenuates tissue fibrosis, CKD progression, and vascular calcification.[22,48] In our study, PWV remained unchanged from 8 wk to 1 y posttransplant, and higher PWV was associated with mortality. It is possible that the interval between PWV measurements is too short and that the arterial stiffness associated with ESRD might resolve some after a longer period of follow-up posttransplant. Another possibility is that the arterial stiffness associated with CKD is irreversible, remaining as a continuous risk factor for CVD in the posttransplant period.

The kidney is the main source of Klotho, and the Klotho levels are low in CKD patients but still present.[49] Exogenous Klotho supplementation may be a new therapeutic approach to delay CKD progression and CVD development in CKD patients and transplant recipients. So far, it has only been tested successfully in animal models.[50] The development of a Klotho-mimetic to maintain or elevate serum Klotho levels could be a future nephroprotective therapeutic option,[51] but currently, a well-functioning graft is the best prognostic marker for kidney transplant recipients.

Recently, mineralocorticoid receptor activation has emerged as a mediator for cardiovascular injury and CKD progression, in addition to Klotho deficiency and FGF23 excess. Mice exposed to mineralocorticoid deoxycorticosterone acetate displayed elevated FGF23 levels, hypertension, and end-organ damage.[52] These changes were reversed by the administration of the mineralocorticoid receptors—antagonists spironolactone and eplerenone.[52] In animal models, hyperphosphatemia was associated with upregulated aldosterone synthase, which is the terminal enzyme in the aldosterone biosynthesis pathway, leading to elevated aldosterone levels due to high phosphate levels.[53] Aldosterone inhibition using an aldosterone antagonist or an aldosterone synthase inhibitor may modify vascular calcification and are possible candidates for improving cardiovascular outcomes in patients with CKD and kidney transplant recipients. There is an intricate interaction between increased iFGF23, reduced sKlotho, suboptimal vitamin D levels, increased aldosterone, and several other factors interacting with the progression of CVD in kidney transplant recipients.

Limitations

The patients were all included at the same time after transplantation, which is a strength in the comparability of the patients and the results. The sample size is a limitation, and it is difficult to draw definite conclusions because of relatively low power, and the results only show trends. The number of events (graft loss or death) is low, and we pooled the 2 events in the survival analyses. Vitamin D, iFGF23, and sKlotho were only measured twice, and a longitudinal profile may have added additional information.

CONCLUSION

In this study, patients with vitamin D deficiency (<30 nmol/L) at 8 wk posttransplant showed reduced graft- and patient survival compared with the patients having higher vitamin D levels posttransplant. iFGF23 and sKlotho measured 8 wk posttransplant were not associated with graft- and patient survival.

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