RESEARCH

Cholecalciferol supplementation effectively improved tertiary hyperparathyroidism, FGF23 resistance and lowered coronary calcification score: a prospective study

Shu-Meng Hu[1], **Yang-Juan Bai**[2], **Ya-Mei Li**[2], **Ye Tao**[1], **Xian-Ding Wang**[3], **Tao Lin**[3], **Lan-Lan Wang**[2] and **Yun-Ying Shi**[1]

[1]Department of Nephrology, West China Hospital, Sichuan University, Chengdu, Sichuan, China
[2]Department of Laboratory Medicine/Research Centre of Clinical Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China
[3]Department of Urology/Institute of Urology, West China Hospital, Sichuan University, Chengdu, Sichuan, China

Correspondence should be addressed to Y-Y Shi: yyshi0130@163.com

Abstract

Introduction: Tertiary hyperparathyroidism (THPT) and vitamin D deficiency are commonly seen in kidney transplant recipients, which may result in persistently elevated fibroblast growth factor 23 (FGF23) level after transplantation and decreased graft survival. The aim of this study is to evaluate the effect of vitamin D supplementation on THPT, FGF23-alpha Klotho (KLA) axis and cardiovascular complications after transplantation.
Materials and methods: Two hundred nine kidney transplant recipients were included and further divided into treated and untreated groups depending on whether they received vitamin D supplementation. We tracked the state of THPT, bone metabolism and FGF23–KLA axis within 12 months posttransplant and explored the predictors and risk factors for intact FGF23 levels, KLA levels, THPT and cardiovascular complications in recipients.
Results: Vitamin D supplementation significantly improved FGF23 resistance, THPT and high bone turnover status, preserved better graft function and prevented coronary calcification in the treated group compared to the untreated group at month 12. The absence of vitamin D supplementation was an independent risk factor for THPT and a predictor for intact FGF23 and KLA levels at month 12. Age and vitamin D deficiency were independent risk factors for coronary calcification in recipients at month 12.
Conclusion: Vitamin D supplementation effectively improved THPT, FGF23 resistance and bone metabolism, preserved graft function and prevented coronary calcification after transplantation.

Key Words

- kidney transplantation
- fibroblast growth factor 23
- alpha Klotho
- vitamin D
- secondary hyperparathyroidism
- coronary calcification

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Introduction

Secondary hyperparathyroidism (SHPT) is one of the common complications that occurs among uraemic patients and is expected to be corrected after kidney transplantation (KT). However, long-lasting SHPT leads to a shift in the growth of parathyroid cells from a polyclonal to a monoclonal manner, which forms parathyroid nodules that lack vitamin D receptor and calcium-sensing receptor and secrete autonomously (1). The autonomous secretion of parathyroid hormone (PTH) is defined as tertiary hyperparathyroidism (THPT), which is characterized by hypercalcaemia and hypophosphatemia (2). According to previous studies, THPT still existed in 15–50% of the kidney transplant recipients (KTRs) (3, 4) and was reported to be closely associated with bone fracture, graft loss, cardiovascular events and all-cause death after KT (2, 3, 4).

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Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone produced by osteocytes and osteoblasts. By binding to FGF receptor 1 (FGFR1), FGF23 activates its canonical signalling pathway and induces the degradation and internalization of sodium-dependent transport protein 2A, which leads to the decrease of phosphate reabsorption, thus promoting the fractional excretion of phosphate (5). alpha Klotho (KLA) is a newly discovered antiaging protein mainly synthesized by kidney distal convoluted tubules and excreted by glomerular filtration (6). While the binding affinity of FGF23 to FGFR1 is rather low, KLA can significantly improve the binding affinity by forming a complex with FGFR1 and therefore is vitally important for FGF23 to exert biological effects (7, 8).

The regulation of FGF23 is complex. Elevated serum phosphate levels can directly elicit the expression of *FGF23* (5, 9). In addition, PTH and vitamin D can both transactivate *FGF23* expression (5, 9). Conversely, FGF23 can inhibit the synthesis of calcitriol (5, 9) and the transcription of the *PTH* gene (10, 11). However, studies have demonstrated that feedback regulation was disrupted under the uraemic milieu, with the expression of *FGFR1* and *KLA* remarkably downregulated in the nodular hyperplasia of parathyroid glands (1, 12), which consequently led to FGF23 resistance in the parathyroid and induced persistent and refractory hyperparathyroidism (13). Studies also reported that massively elevated FGF23 was able to target cells in an KLA-independent way and cause a series of pathological changes, including cardiac hypertrophy, myocardial fibrosis (14, 15, 16), abnormal expression of cytokines and disordered immunocyte function (17, 18, 19).

Although vitamin D synthesis defects are supposed to be ameliorated by KT, vitamin D deficiency persists in most KTRs (20). Previous studies have suggested that vitamin D supplementation may help attenuate hyperparathyroidism and prevent bone disease (21, 22). However, very few studies have focused on the effect of vitamin D supplementation on the THPT, FGF23–KLA axis and coronary lesions at an early stage after KT. The primary objective of this study was to evaluate the effects of vitamin D supplementation on THPT, the FGF23-KLA axis, graft function and coronary calcification within the first 12 months after transplantation.

Methods and materials

Description of the cohort

KTRs who underwent their first kidney transplant in West China Hospital between June 2020 and December 2020 were recruited as a prospective cohort to study the impacts of vitamin D supplementation on THPT, the FGF23–KLA axis and coronary calcification after KT. The inclusion criteria were as follows: (i) recipients aged from 18 to 65 years; (ii) recipients receiving their first KT with regular follow-ups at West China Hospital; and (iii) PTH level >65 pg/mL within 1 month posttransplant. The exclusion criteria included recipients with (i) acute rejection and infection occurring within 4 weeks prior to vitamin D supplementation; (ii) delayed graft function; (iii) parathyroidectomy before KT; (iv) ABO-incompatible transplantation; (v) history of malignancy or autoimmune diseases; and (vi) primary parathyroid disease. All KTRs received basiliximab for induction therapy following standard triple immunosuppressive maintenance therapy consisting of tacrolimus (Tac), mycophenolate mofetil and corticosteroids. Healthy adults who had physical examinations at West China Hospital were also included in the healthy control (HC) group, and adult patients under 65 with chronic kidney disease (CKD) stage 3 were included in the CKD group. This study was approved by the Ethics Committee of West China Hospital, and written informed consent was obtained from each participant before enrolment. The study was preregistered in the Chinese Clinical Trial Registry, and the register number is ChiCTR2200056077.

Data collection and measurements

General demographic information and pretransplant history were collected for all study participants. All KTRs were regularly followed up, and immunosuppression medication was strictly monitored by physicians in the West China Hospital.

Fasting blood samples of KTRs were collected in the morning at pretransplant and at 2 weeks, 3 months, 6 months and 12 months posttransplant for biochemical testing. Fasting blood samples of CKD patients and HC were collected at the same cross-sectional time points. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. Routine examinations, including complete blood count, serum creatinine (SCr), serum calcium, serum inorganic phosphate, urinary calcium and PTH, as well as bone turnover markers (BTMs), including bone-specific alkaline phosphatase (bALP), N-terminal propeptide of type 1 collagen (P1NP), type I collagen carboxyl-terminal peptide (BCTX), and N-MID osteocalcin (NMID), were measured in the central laboratory of the West China Hospital. Chest routine scan and coronary

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calcification score (CACS) on a dual-source CT scanner (Syngo CaScore; Siemens, Forchheim, Germany) were obtained, and images were also reconstructed with 0.6-mm and 3.0-mm slice thicknesses for each patient. The Agatston scoring method was applied to the reconstructed image set by commercially available software (Syngo CaScore) to obtain the CACS for each patient at month 12. Serum concentrations of intact FGF23 (iFGF23) (Boster Biological Technology., Ltd., Wuhan, China) and KLA (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) were measured using ELISA methods with the available commercial kits. The serum concentration of 25 hydroxyvitamin D (25(OH)D) was measured by HPLC/mass spectrometry. Cardiovascular events were obtained by phone calls to patients' homes and by reviewing the hospital records. In the study, we defined hypercalcaemia and hypophosphatemia as serum calcium ≥2.52 mmol/L and phosphataemia ≤0.85 mmol/L, respectively. Hyperparathyroidism was defined as PTH ≥65 pg/mL. 25(OH)D status was defined according to Holick's definition: 25(OH)D > 75 nmol/L indicated vitamin D sufficiency, and 25(OH)D ≤ 75 nmol/L indicated vitamin D deficiency (20).

Hyperparathyroidism treatment strategy

Since not every physician held the same opinion on the necessity of early treatment of hyperparathyroidism, whether the KTRs included received treatment for hyperparathyroidism was dependent on the patients' and physicians' own will. The detailed hyperparathyroidism treatment strategy was based on the levels of 25(OH)D, serum calcium and PTH. Cholecalciferol was administered to KTRs at an initial dose of 2000 international units (IU) per day to correct vitamin D deficiency, and the levels of 25(OH)D were monitored once every 3 months thereafter. The cholecalciferol doses were cut to 400–800 IU/day once KTRs had a sufficient 25(OH)D level. For KTRs who still had hyperparathyroidism but no vitamin D deficiency, calcitriol was then added to control hyperparathyroidism (Fig. 1). Serum calcium levels were monitored during the follow-up. In KTRs with hypercalcaemia, calcitriol would be stopped to remit hypercalcaemia, and cholecalciferol would be cut to 400 IU/day if vitamin D deficiency existed persistently. KTRs with consistently elevated serum calcium levels (≥2.6 mmol/L) were given cinacalcet, and single-photon emission CT of the parathyroid glands was arranged and then discussed with the hyperparathyroidism multidisciplinary team for further treatment, such as parathyroidectomy or radiofrequency ablation.

Statistical analysis

Normality tests of continuous variables were performed with the Shapiro–Wilk test. Variables with a normal distribution are displayed as the mean ± S.D. and were compared using independent samples *t*-test or ANOVA. Asymmetric variables are reported as the median (interquartile range), and comparisons were assessed with the Mann–Whitney *U* test and Kruskal–Wallis test. Categorical variables were compared using the chi-square test or Fisher's exact test. Multiple linear regression was used to explore the potential correlates of postoperative iFGF23 and KLA levels. Potential risk factors for THPT and positive findings in CACS after KT were explored using logistic regression models, and the number of bootstraps was 500. A nomogram to predict THPT and CACS at 12 months after KT was constructed using stepwise selection and the Akaike information criterion (AIC). The stepwise selection method was used to select variables and determine model fit. AIC was used for model evaluation and model selection. A receiver operating characteristic (ROC) curve and area under the receiver operating characteristic curve (AUROC) were used to analyse the predictive performance of the model. All the reported *P* values are two-tailed, and $P<0.05$ was considered statistically significant for the tests above. All statistical analyses were performed using Empower® (www.empowerstats.com, X&Y Solutions Inc., Boston MA) and SPSS version 28.0 statistical software (SPSS Company).

Results

Description of the study population

A total of 255 patients received KT at West China Hospital between June 2020 and December 2020, among whom 212 adult KTRs met the inclusion criteria and were recruited. Two KTRs died from severe infection, and one KTR underwent parathyroidectomy within 12 months posttransplant. A total of 209 KTRs were included in the KT group. The treatment strategy for THPT in the KT group is shown in Fig. 1. The characteristics of KTRs and the specific regimens of vitamin D supplementation are shown in Supplementary Table 1 (see section on supplementary materials given at the end of this article). The Tac concentration was controlled within 5.95–7.11 ng/mL, and the area under the curve of mycophenolate acid was controlled within 45.7–70.9 mg·h/L during the follow-up. Vitamin D supplementation was initiated at 2 (1-4) weeks posttransplant in the treated group.

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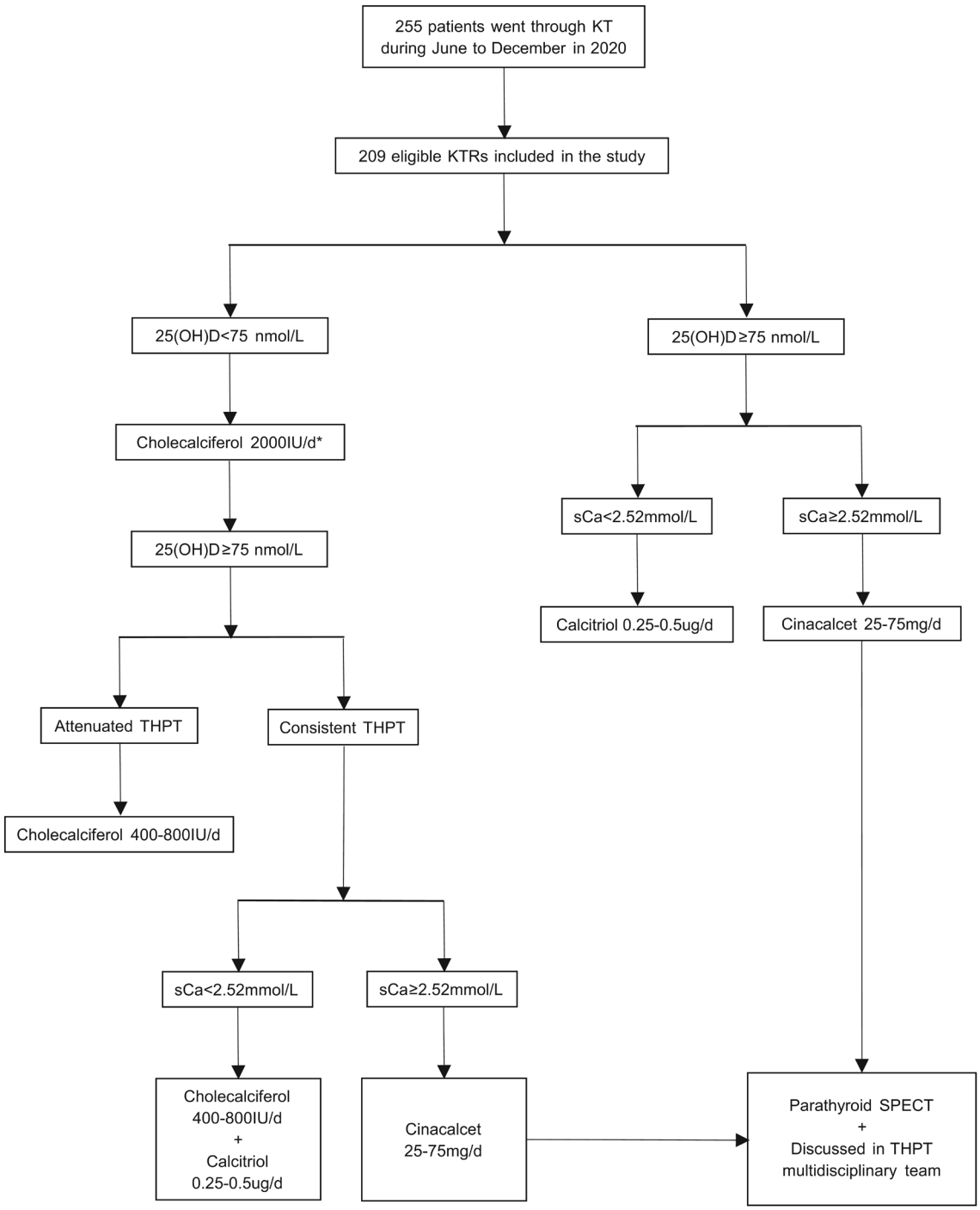

Figure 1
THPT treatment strategy for KTRs included in the present study. *If hypercalcemia is observed in KTRs with vitamin D deficiency during follow-up, the dose of cholecalciferol will be cut from 2000–400 IU/day to remit hypercalcemia. KTRs with both vitamin D deficiency and consistent hypercalcemia will be given cinacalcet to lower the serum calcium level.

Forty-eight healthy adults and 46 patients with stage 3 CKD were included in the study as the HC group and CKD group, respectively (Supplementary Table 2). No significant difference was found in age, sex ratio or calcium level among the three groups. Although the eGFR level in the KT group was much higher than that in the CKD group, the KT group still had significantly higher levels of iFGF23 and PTH at week 2 than the CKD group.

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Changes in biochemical parameters in KTRs

The graft function and laboratory parameters of the KT group at pretransplant, week 2, month 3, month 6 and month 12 and the comparison between the KT group and the HC/CKD group are displayed in Table 1. A total of 118 (56.46%) and 108 (51.67%) KTRs still had hyperparathyroidism at month 6 and month 12, respectively.

In accordance with that in previous studies, we observed stable graft function and a gradually improved state of THPT in all 209 KTRs. The levels of serum calcium and phosphate tended to be normal, and abnormal BTM levels were improved (Table 1). Meanwhile, the levels of PTH and iFGF23 decreased significantly within 12 months posttransplant, and the levels of 25(OH)D and KLA increased continuously and significantly within 12 months posttransplant (Table 1).

Effects of vitamin D supplementation on laboratory parameters, graft function and CACS

A total of 209 KTRs were further divided into a treated group (108 KTRs) and an untreated group (101 KTRs) according to whether they received vitamin D supplementation. No significant difference was noted in baseline parameters between the two groups (Supplementary Table 3). The number of KTRs with coronary calcification examined by chest routine scanning (CT) showed no difference between the two groups (Supplementary Table 3).

The serum calcium levels at month 3, month 6 and month 12 were significantly higher in the untreated group than in the treated group (Fig. 2A and Tables 2, 3). The serum phosphate level in the treated group rose above the lower limit after month 3 and was significantly higher than that in the untreated group at month 6 and month 12 (Fig. 2B and Tables 2, 3). The PTH level in the treated group was significantly lower than that in the untreated group after month 3 (Fig. 2C). Since KTRs in the treated group received vitamin D supplementation, the 25(OH)D levels since month 3 were significantly higher than those in the untreated group (Fig. 2D). At month 6 and month 12, the prevalence rates of KTRs with hypercalcaemia, hypophosphatemia and hyperparathyroidism in the untreated group were found to be dramatically higher than those in the treated group (Tables 2 and 3).

The iFGF23 levels in the treated group were significantly lower after month 3 than those in the untreated group (Fig. 3A and Tables 2, 3). While KTRs in the untreated group also had an iFGF23 level equivalent to that in the CKD group, the iFGF23 level in the treated group showed no difference compared to that in the HC group at month 6 and remained the same at month 12. The levels of KLA were significantly higher in the treated group after month 6 than in the untreated group (Fig. 3B and Tables 2, 3). However, the KLA levels in both groups were significantly higher than those in the CKD group after month 3 (Fig. 3B).

For BTM, BCTX and P1NP levels decreased dramatically at week 2 after the kidney transplant and then gradually increased ever since. With vitamin D supplementation, significantly lower BCTX and P1NP levels were noted in the treated group than in the untreated group since month 6 posttransplant (Fig. 3C, D and Tables 2, 3).

A difference was not observed in graft function between the two groups until month 12, as the treated group had a significantly lower creatinine level and a higher eGFR level at month 12 than the untreated group (Fig. 2E and Table 3).

At 12 months, the CACS in the untreated group significantly exceeded that in the treated group, and the number of KTRs with CACS>0 was significantly higher in the untreated group than in the treated group (Table 3). However, no cardiovascular events were observed in any of the 209 KTRs within 12 months posttransplant.

Correlations between vitamin D treatment and THPT, iFGF23, KLA and CACS

To identify the risk factors for hyperparathyroidism and CACS >0 at month 12 after KT, we performed univariate regression analyses. As shown in Table 4, sex, FGF23 levels at week 2 and vitamin D supplementation before and after KT were associated with the odds of THPT at month 12. Age, coronary calcification before KT and vitamin D supplementation before and after KT were associated with positive findings on the CACS. Further multivariate logistic regression including the variables above identified sex and vitamin D supplementation before and after KT as the final variables for the final logistic regression model for THPT at month 12. Age and vitamin D supplementation before and after KT were selected as the final logistic regression models for CACS >0 at month 12. All variables were selected based on stepwise selection and AIC values (Table 4). The results showed that female sex and the absence of vitamin D supplementation before and after transplantation were three independent risk factors for persistent hyperparathyroidism at month 12. Male KTRs were associated with an 88.9% (95% CI: 0.014–0.902, P = 0.004) decreased risk of persistent THPT at month 12, while KTRs without vitamin D supplementation before and after KT were associated with 2.269

Table 1 Change of laboratory parameters in KT group at four time points (n = 209).

	Preoperation	2 weeks after KT	3 months after KT	6 months after KT	12 months after KT
SCr (μmol/L)	1053.71 ± 298.02[e,f]	121.58 ± 35.62[a,e,f]	116.99 ± 29.25[a,e,f]	119.10 ± 32.50[a,e,f]	118.85 ± 34.95[a,e,f]
eGFR (mL/min/1.73m^2)	4.86 ± 1.41[e,f]	68.69 ± 23.61[a,e,f]	67.20 ± 15.62[a,e,f]	67.05 ± 21.67[a,e,f]	65.34 ± 13.12[a,e,f]
Mean trough level of Tac (ng/mL)	-	5.87 ± 1.46	7.12 ± 1.19[b]	6.70 ± 1.23[b]	6.82 1.16[b]
Calcium (mmol/L)	2.24 ± 0.23[e,f]	2.31 ± 0.14[a]	2.48 ± 0.17[a,b,e,f]	2.47 ± 0.18[a,b,e,f]	2.46 ± 0.15[a,b,e,f]
Hypercalcemia	22 (10.53%)	9 (4.31%)	98 (46.89%)[a,b]	89 (42.58%)[a,b]	84 (40.19%)[a,b]
Phosphate (mmol/L)	1.91 ± 0.45[e,f]	0.60 ± 0.22[a,e,f]	0.82 ± 0.24[a,e,f]	0.91 ± 0.19[a,e,f]	1 ± 0.31[a,b,e,f]
Hypophosphatemia	8 (3.83%)	186 (89.00%)[a]	125 (59.81%)[a,b]	109 (52.15%)[a,b]	103 (49.28%)[a,b]
Ca×Pi Product (mg^2/dL^2)	53.05 ± 14.62[e,f]	17.19 ± 8.62[a,e,f]	25.22 ± 6.50[a,b,f]	27.87 ± 6.32[a,b,f]	30.56 ± 9.48[a,b,c,f]
PTH (pg/mL)	449.55 (224.24–929.27)[e,f]	192.47 (113.42–301.30)[a,e,f]	150.61 (103.29–216.66)[a,e,f]	86.00 (64.75–169.74)[a,b,c,e,f]	94 (54.5–162))[a,b,c,e,f]
Hyperparathyroidism	200 (96.33%)	197 (94.26%)	175 (83.73%)	118 (56.46%)[a,b,c]	108 (51.67%)[a,b,c]
25(OH)D (nmol/L)	61.85 (31.51–101.49)[f]	41.30 (28.60–53.13)[a]	57.79 (41.02–68.48)[b]	76.99 (49.81–87.54)[b,f]	78 (63.75–89)[b,c,f]
Vitamin D deficiency	117 (55.98%)	177 (84.69%)[a,e,f]	156 (74.64%)[a,e,f]	104 (49.76%)[b,c,e,f]	78 (37.32%)[a,b,c,d,e,f]
bALP (μg/L)	15.8 (11.43–26.21)	13.57 (10.57–16.04)	17.42 (14.37–47.36)[b]	16.29 (13.56–25.05)[b]	19 (11.75–25)[b]
P1NP (ng/mL)	313 (287–449.5)	58.1 (45.8–83.9)[a]	101 (40.9–159.5)[a,b]	103.65 (54.93–185)[a,b]	62.5 (39.75–108)[a,b,c,d]
BCTX (ng/mL)	3.61 ± 1.66	1.12 ± 0.91[a]	1.36 ± 1.30[a]	1.37 ± 0.93[a,b]	0.91 ± 0.38[a,b,c,d]
NMID (ng/mL)	210.71 ± 68.43	46.05 ± 29.72[a]	36.60 ± 14.20[a,b]	31.87 ± 16.41[a,b]	25.77 ± 10.4[a,b,c,d]
FGF23 (pg/mL)	11600 (4265.83–12000)[e,f]	623.79 (139.58–1597.17)[a,e,f]	106.00 (86.00–139.67)[a,b,e]	67.33 (43.00–117.00)[a,b,e,f]	53.93 (30.45–86.5)[a,b,c,d,e,f]
KLA (pg/mL)	412 (285.5–510)[e,f]	392 (305–442.75)[a,e,f]	504 (380.5–817)[b,e,f]	723.5 (503.5–878)[a,b,c,e,f]	898 (617.5–1098)[a,b,c,d,e,f]

[a]P < 0.05 compared with preoperation. [b]P < 0.05 compared with week 2. [c]P < 0.05 compared with month 3. [d]P < 0.05 compared with month 6. [e]P < 0.05 compared with HC. [f]P < 0.05 compared to CKD group.

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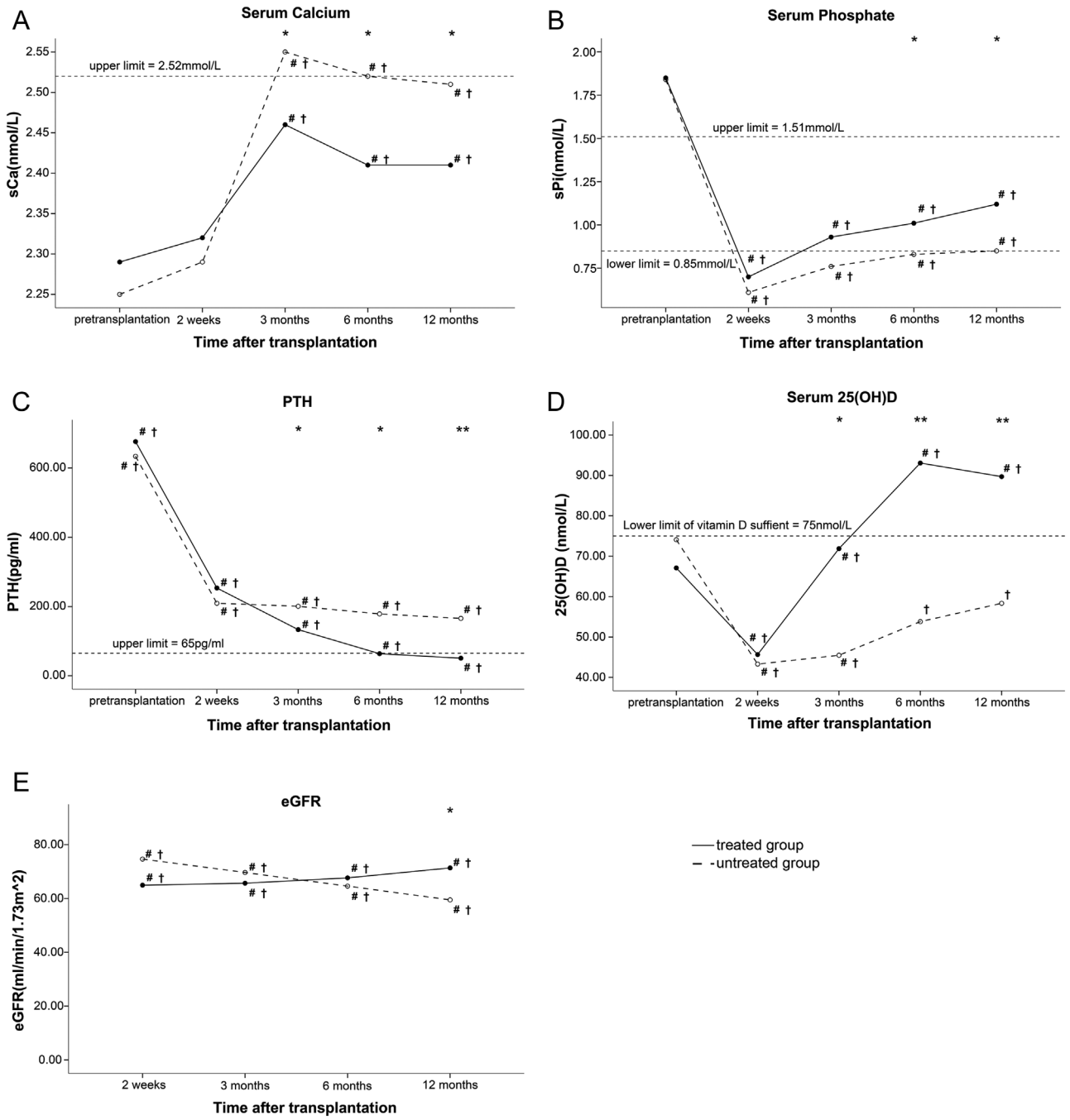

Figure 2

Natural history of (A) serum calcium, (B) serum phosphate, (C) PTH, (D) 25(OH)D and (E) eGFR in treated and untreated group within 12 months posttransplant. *P < 0.05 compared between groups. **P < 0.01 compared between groups. $^{\#}P$ < 0.05 compared with the HC group. $^{\dagger}P$ < 0.05 compared with the CKD group.

(95% CI: 1.246–4.129, P = 0.007) and 2.418 (95% CI: 1.343–4.356, P = 0.003) higher risks of persistent THPT at month 12, respectively. For CACS, logistic regression showed that age and the absence of vitamin D supplementation before and after KT were independent risk factors for coronary calcification at month 12. Every 1-year increase was associated with a 1.205 higher risk (95% CI: 1.156–1.269, P > 0.001), and the absence of vitamin D supplementation before and after KT was associated with 2.144 (95% CI: 0.941–4.885, P = 0.003) and 3.540 (95% CI: 1.315–9.530, P = 0.012) higher risks of CACS >0 at month 12. Based on the multivariate logistic regression analysis, a prognostic nomogram (Figs 4 and 5) for predicting the occurrence of hyperparathyroidism and CACS at month 12 was built.

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Table 2 Comparison of laboratory parameters between vitamin D-treated group and -untreated group at month 6 (n = 209).

	Treated Group (n = 108)	**Untreated Group** (n = 101)	***P***
SCr (μmol/L)	125.67 ± 41.31	112.24 ± 24.96	0.192
eGFR (mL/min/1.73 m^2)	66.14 ± 24.82	64.58 ± 23.13	0.833
Calcium (mmol/L)	2.45 (2.33–2.48)	2.54 (2.38–2.62)	0.011[a]
Hypercalcemia	14 (12.96%)	75 (74.26%)	0.000[a]
Phosphate (mmol/L)	1.01 ± 0.23	0.83 ± 0.17	0.014[a]
Hypophosphatemia	31 (28.70%)	78 (77.23%)	0.000[a]
Ca×Pi product (mg^2/dL^2)	29.52 ± 6.38	25.75 ± 6.78	0.095
PTH (pg/mL)	63.74 ± 13.21	178.70 ± 81.18	0.000[a]
Hyperparathyroidism	26 (24.07%)	92 (91.09%)	0.000[a]
25(OH)D (nmol/L)	96.09 ± 26.77	53.81 ± 21.97	0.004[a]
Vitamin D deficiency	14 (12.96%)	90 (89.11%)	0.000[a]
FGF23 (pg/mL)	42.00 (37.00–56.17)	103.00 (76.63–163.08)	0.001[a]
KLA (pg/mL)	803 (470.5–925)	680 (523-815)	0.041[a]
bALP (ug/L)	18.41 (12.86–30.74)	17.98 (14.41–25.43)	0.179
P1NP (ng/mL)	55.14 (37.34–93.65)	178.27 (124.00–231.68)	0.000[a]
BCTX (ng/mL)	0.94 ± 0.80	1.78 ± 0.87	0.001[a]
NMID (ng/mL)	33.93 ± 21.14	29.96 ± 10.48	0.423

[a]$P < 0.05$ compared with treated group.

Internal validation was performed via the bootstrapping method (number of resamples: 500), and the ROC curve was generated, with satisfactory AUROCs of 0.668 (95% CI: 0.596–0.740) and 0.897 (95% CI: 0.856–0.938) for hyperparathyroidism and CACS at month 12, respectively (Fig. 6).

The results of the multivariate linear analysis demonstrated that female sex (β = 27.85; P = 0.0168) and vitamin D supplementation after transplantation (β = −7.8; P < 0.0001) were independent predictors of iFGF23 levels at month 12, indicating that the application of vitamin D after transplantation was correlated with a 7.8 pg/mL decrease in iFGF23 levels. Vitamin D supplementation at month 12 (β = 209.49; P = 0.046) was found to be the only predictor for the KLA level at month 12, indicating that vitamin D supplementation was associated with a 209.49 pg/mL increase in serum soluble KLA at month 12 in KTRs.

Discussion

In this study, we observed hyperparathyroidism and vitamin D deficiency in more than half of the KTRs at

Table 3 Comparison of laboratory parameters and CACS between vitamin D-treated group and -untreated group at month 12 (n = 209).

	Treated Group (n = 108)	**Untreated Group** (n = 101)	***P***
SCr (μmol/L)	114.28 ± 32.53	123.41 ± 37	0.000[a]
eGFR (mL/min/1.73 m^2)	71.34 ± 10.57	59.47 ± 12.79	0.000[a]
Calcium (mmol/L)	2.41 ± 0.14	2.51 ± 0.15	0.011[a]
Hypercalcemia	12 (11.11%)	72 (71.29%)	0.000[a]
Phosphate (mmol/L)	1.12 ± 0.27	0.85 ± 0.25	0.014[a]
Hypophosphatemia	23 (21.29%)	80 (79.21%)	0.000[a]
Ca×Pi product (mg^2/dL^2)	35.39 ± 8.73	25.73 ± 7.61	0.000[a]
PTH (pg/mL)	50.80 ± 25.91	165.83 ± 44.63	0.000[a]
Hyperparathyroidism	19 (17.59%)	89 (88.12%)	0.000[a]
25(OH)D (nmol/L)	89.7 ± 16.00	58.35 ± 24.15	0.004[a]
Vitamin D deficiency	10 (9.23%)	68 (67.33%)	0.000[a]
FGF23 (pg/mL)	30.30 (24.16–37.50)	87.00 (74.83–109.50)	0.000[a]
KLA (pg/mL)	961.00 (752.5–1133)	791.00 (485–1045.5)	0.000[a]
CACS	1.16 (0–9.59)	6.9 (0.63–133.99)	0.016[a]
CACS >0	60 (55.56%)	75 (74.26%)	0.005[a]
bALP (μg/L)	21.5 (12–28)	19 (11.25–22)	0.144
P1NP (ng/mL)	40.28 ± 15.15	109.07 ± 30.21	0.000[a]
BCTX (ng/mL)	0.62 (0.49–0.84)	1.27 (0.99–1.37)	0.033[a]
NMID (ng/mL)	29 (20–36.75)	22.5 (14–30.75)	0.097

[a]$P < 0.05$ compared with treated group.

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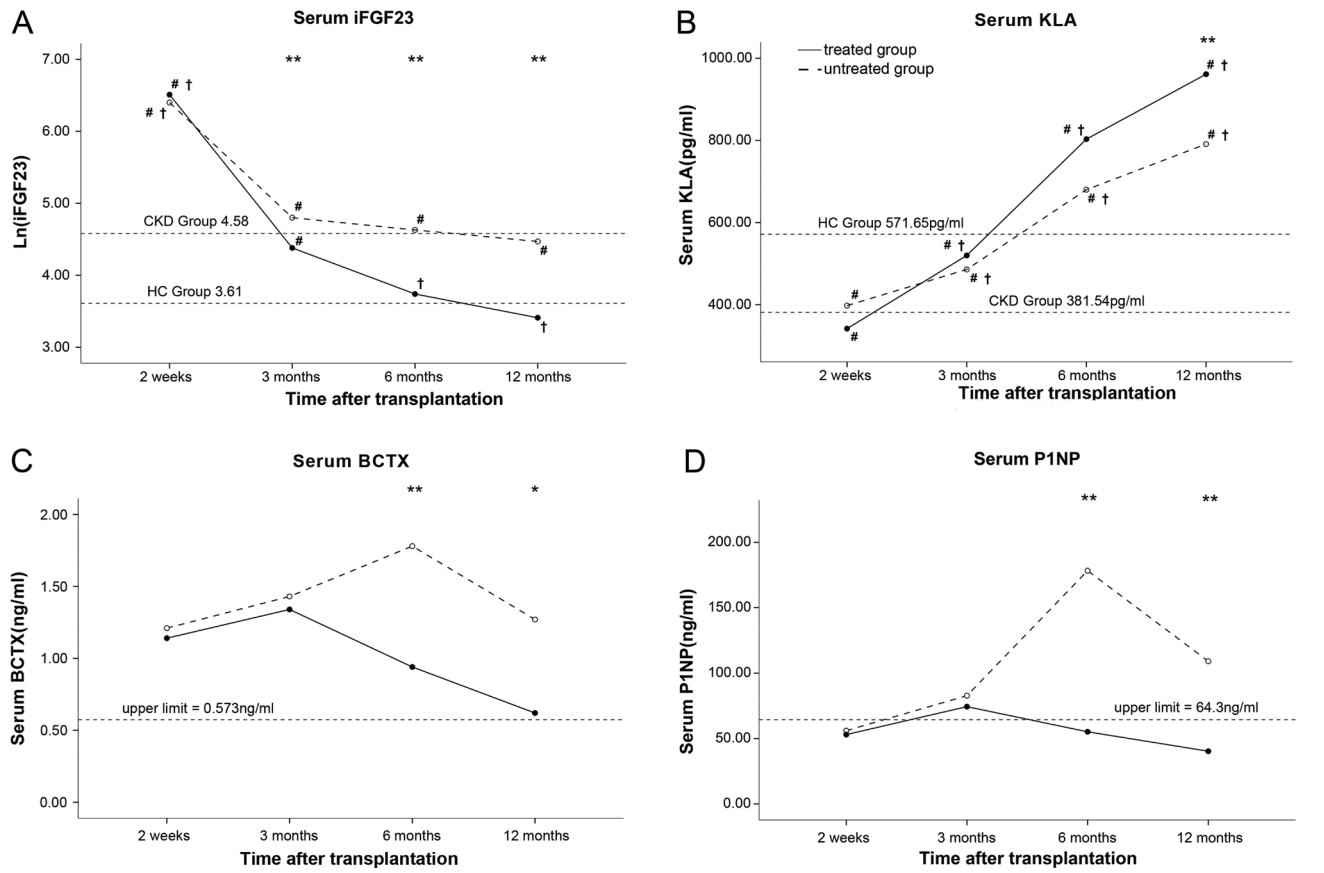

Figure 3
Change of (A) iFGF23 (logarithmic scale; Ln iFGF23), (B) KLA, (C) BCTX and (D) P1NP in treated and untreated group within 12 months posttransplant. *P < 0.05 compared between groups. **P < 0.01 compared between groups. $^{\#}P$ < 0.05 compared with HC group. $^{\dagger}P$ < 0.05 compared with CKD group.

week 2, as well as decreased phosphate levels, elevated calcium levels and active bone turnover caused by THPT and continuous FGF23 secretion posttransplant. These results were consistent with those of previous studies (23, 24, 25). Compared with the treated group, the prevalence of THPT in the untreated group barely improved if no intervention was administered within 12 months posttransplant (Tables 1, 2 and 3). Although the KT group had higher eGFR levels than the CKD group, the PTH levels in the KT group still dramatically exceeded those in the CKD group even at month 12 (Table 1). Our data proved that hyperparathyroidism would not be spontaneously ameliorated after KT and would persistently affect bone mineral metabolism and the function of the FGF23–KLA axis in KTRs with well-recovered graft function and that cholecalciferol supplementation after KT could improve hyperparathyroidism and bone mineral metabolism. In our cohort, most KTRs experienced a hyperparathyroidism with hypercalcaemia and/or hypophosphatemia, indicating the occurrence of THPT. In THPT, nodular hyperplastic parathyroid cells express fewer vitamin D receptors (VDRs) and calcium-sensing receptors, thus leading to uncontrolled PTH secretion with no response to the suppression by vitamin D or calcium (1), which seems to conflict with our findings. The explanation may exist in the fact that the pathological changes of the parathyroid gland in the early stage after KT might be a mixture of polyclonal and monoclonal hyperplasia of parathyroid tissue, therefore the response to the regulation of vitamin D was partially preserved. Several studies reported that up to 29% of KTRs with THPT had only one or two nodular hyperplastic glands (26). However, data about the pathological changes of parathyroid glands in the early posttransplant period are scarce given that surgical intervention of mild THPT after KT is recommended to be assessed and carried out after 12 months posttransplant when graft function is stable (27).

Studies in various regions reported that the prevalence of vitamin D deficiency was above 50% in both short-term and long-term KTRs (28). In another study of KTRs in the northern region, 45% of 334 KTRs were found to have vitamin D deficiency, even if they were given oral vitamin D

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Table 4 Multivariate regression analysis of risk factors for THPT and CACS at month 12.

Clinical factor	Unvariate regression			Multivariate regression		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Risk factors for SHPT at month 12						
Age	1.009	0.985–1.034	0.476			
Vitamin D supplementation after KT	2.108	1.211–3.669	0.008	2.418	1.343–4.356	0.003[a]
Vitamin D supplementation before KT	2.545	1.363–4.752	0.003	2.269	1.246–4.129	0.007[a]
Gender (male)	0.421	0.241–0.736	0.002	0.889	0.014–0.902	0.004[a]
Dialysis duration	1.036	0.863–1.243	0.706			
BMI	1.079	0.910–1.278	0.381			
DM	1.407	0.619–3.198	0.415			
Donor type (DCD)	0.937	0.522–1.682	0.828			
2 week sCa	0.279	0.057–1.364	0.115			
2 week sPi	0.851	0.433–1.029	0.256			
2 week PTH	1.000	1.000–1.001	0.265			
2 week FGF23	0.999	0.998–1.000	0.011	0.998	0.979–0.999	0.256
2 week 25(OH)D	0.993	0.979–1.008	0.343			
2 week KLA	0.678	0.423–0.985	0.265			
PTH before KT	1.064	0.999–1.139	0.038	1.010	1.000–1.139	0.098
sCa before KT	1.254	0.870–2.634	0.435			
sPi before KT	1.003	0.985–1.050	0.358			
FGF23 before KT	1.254	0.842–3.601	0.522			
Risk factors for CACS >0 at month 12						
Age	1.193	1.136–1.253	0.000	1.205	1.156–1.269	0.000[a]
Vitamin D supplementation after KT	4.580	2.102–9.980	0.004	3.540	1.315–9.530	0.012[a]
Vitamin D supplementation before KT	2.347	1.305–4.219	0.000	2.144	0.941–4.885	0.003[a]
Gender (male)	0.762	0.430–1.349	0.351			
Dialysis duration	1.148	0.989–1.331	0.069			
BMI	0.984	0.825–1.174	0.860			
DM	0.930	0.402–2.151	0.865			
Donor type (DCD)	1.787	0.982–3.254	0.057			
2 week sCa	0.344	0.066–1.791	0.205			
2 week sPi	1.099	0.870–1.372	0.098			
2 week PTH	1.308	0.809–2.411	0.309			
2 week FGF23	1.029	0.828–1.348	0.052			
2 week 25(OH)D	0.991	0.976–1.006	0.246			
2 week KLA	0.989	0.560–1.746	0.969			
Coronary calcification before KT	2.144	1.194–3.851	0.011	3.567	1.549–8.212	0.069
PTH before KT	1.162	0.846–1.534	0.309			
sCa before KT	1.058	0.708–1.695	0.605			
sPi before KT	1.206	1.001–1.601	0.309			
FGF23 before KT	2.331	1.402–3.830	0.002	2.012	1.168–4.541	0.198

[a]Selected in final logistic regression model.

supplementation (29). Our data demonstrated that the proportion of KTRs with vitamin D deficiency increased dramatically at week 2 compared to pretransplant and barely changed thereafter if vitamin D supplementation was not applied (Fig. 1D and Tables 2, 3). Theoretically, the expression of *CYP27B1* increases with the recovery of graft function, which leads to the consumption of 25(OH)D and vitamin D deficiency in a short period of time after KT. However, the shortage of 25(OH)D was difficult to recover without exogenous vitamin D supplementation within 12 months posttransplant in our study.

Previous studies reported that calcitriol improved the expression of the *FGF23* gene *in vitro* (30, 31, 32). However, we found that iFGF23 in the treated group was significantly lowered by vitamin D supplementation compared with that in the untreated group. In vivo, numerous factors work together to regulate iFGF23 levels after transplantation. The increased levels of serum phosphate, serum calcium, serum iron, PTH, calcitriol, inflammation, etc. are all responsible for the elevation in iFGF23 levels (9). Therefore, the decline in iFGF23 levels in KTRs with vitamin D supplementation might be multifactorial. Firstly, since PTH can induce the expression of the *FGF23* gene (33), active vitamin D metabolites may indirectly reduce the expression of the *FGF23* gene by suppressing hyperparathyroidism. Secondly, the expression of several

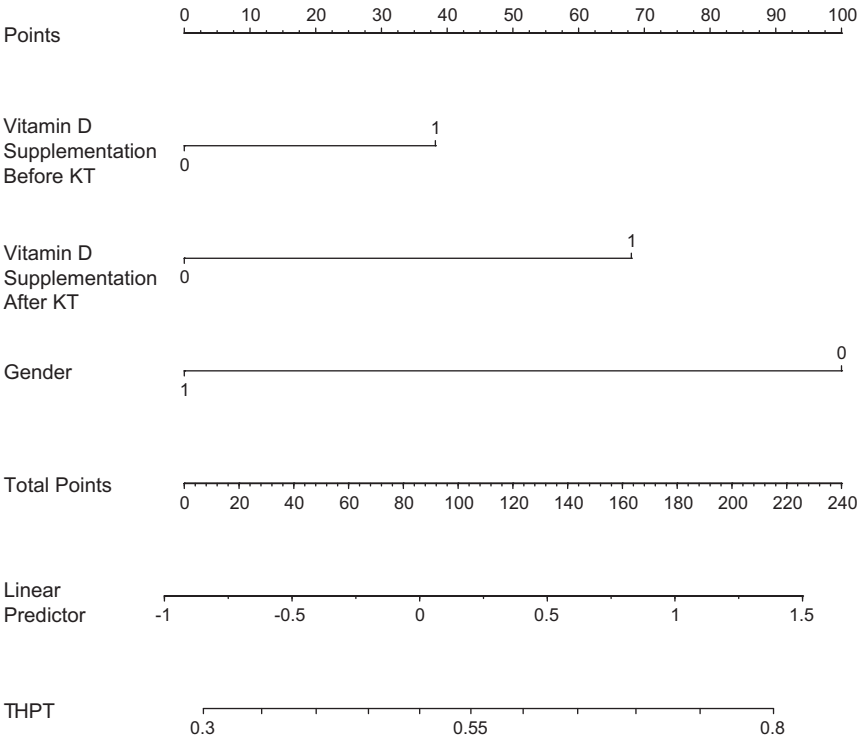

Figure 4
Nomogram for predicting THPT at month 12. The nomogram represents the predicted probability of THPT at month 12 on a scale of 0–240. For each predictor, draw a vertical line straight up to the point axis and note the corresponding points. Sum the points from each predictor, and the total score corresponding to a predicted probability of THPT at month 12 can be found at the bottom of the nomogram.

proinflammatory cytokines, including interleukin 1 (IL1), IL6 and TNF, can induce the expression of the *FGF23* gene directly through nuclear factor KB activation (34) or indirectly by promoting the secretion of hepcidin (35). The immunomodulatory effect of active vitamin D may inhibit the production of proinflammatory cytokines (36) and further lead to decreased expression of the *FGF23* gene. Previous *in vitro* studies have proven that *VDR* and *CYP27B1* are expressed in several innate and adaptive immune cells, which enables immune cells to synthesize active vitamin D

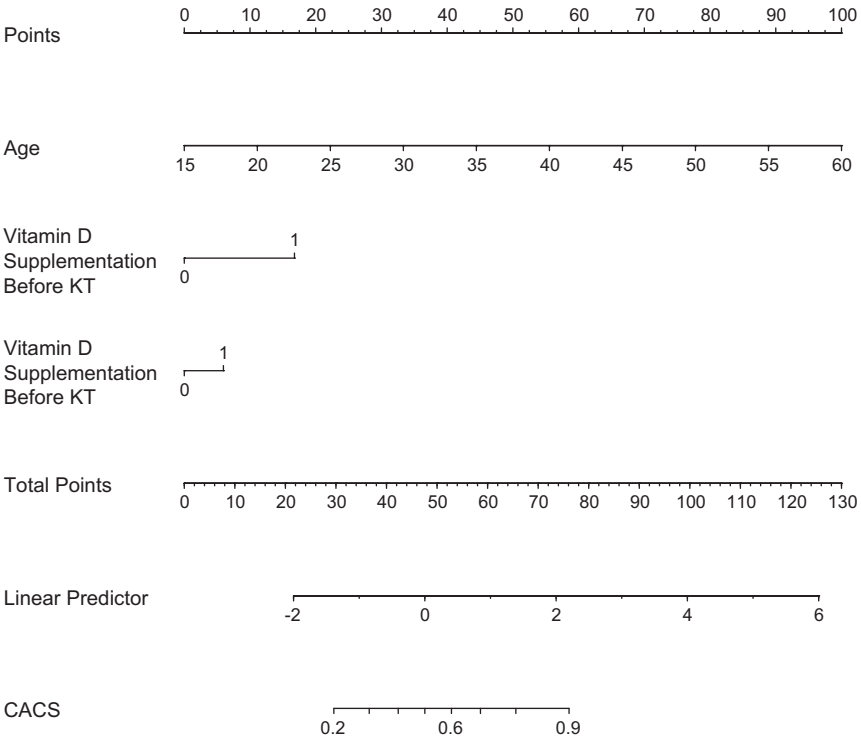

Figure 5
Nomogram for predicting coronary calcification (CACS >0) at month 12. The nomogram represents the predicted probability of coronary calcification on a scale of 0–130. For each predictor, draw a vertical line straight up to the point axis and note the corresponding points. Sum the points from each predictor, and the total score corresponding to a predicted probability of coronary calcification can be found at the bottom of the nomogram.

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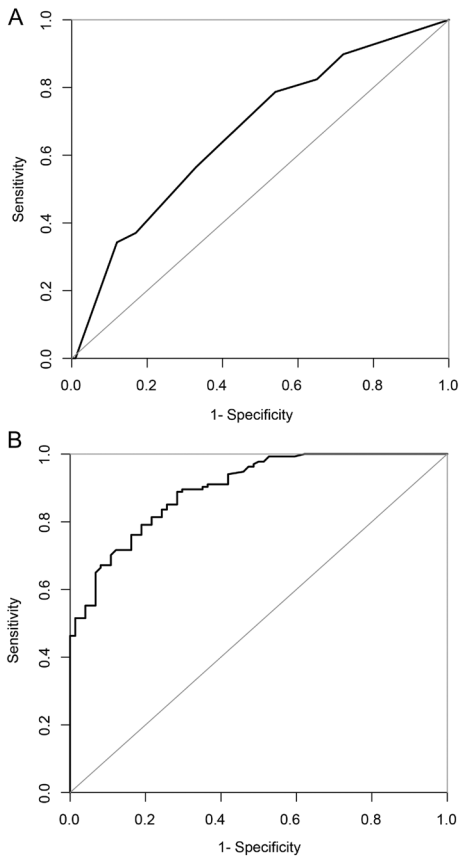

Figure 6
ROC curve for predicting THPT and coronary calcification (CACS >0) at month 12. (A) ROC curve for predicting THPT obtained by the bootstrapping method (resample: 500), with an AUROC of 0.897 (95% CI: 0.856–0.938). (B) ROC curve for predicting coronary calcification obtained by the bootstrapping method (resample: 500), with an AUROC of 0.668 (95% CI: 0.596–0.740).

metabolite and regulate both innate and adaptive immune functions in an autocrine or paracrine manner (36, 37), indicating that correcting vitamin D deficiency with cholecalciferol might maximize the immunomodulatory function of vitamin D.

In our study, vitamin D supplementation after transplantation was found to be closely related to the increase in KLA, which was consistent with the *in vitro* research findings (9). Studies have proven that soluble KLA plays a protective role in ischemia-reperfusion injury and renal fibrosis by regulating the function of several growth factors and ion transporters involved in epithelial–mesenchymal transition and proteinuria. The results of our study indicated that vitamin D supplementation might benefit graft function by elevating the level of soluble KLA, which provides another basis for the application of vitamin D after KT. Vitamin D was reported to directly induce the expression of KLA by binding to VDR and thereafter transactivating the promoter of the *KLA* gene (9). In previous studies, a transient reduction in KLA levels during the acute postoperative phase followed by a consistent increase was generally observed (24, 38, 39), which showed a similar trend to our findings (24). Since the kidney is responsible for both the synthesis and cleavage of soluble KLA, the main reason for the initial decline at week 2 may lie in the increased excretion of soluble KLA and reduced soluble KLA production, which may be caused by the transient dysfunction of renal tubules at the acute postoperative phase. However, correlation analyses in a previous study at month 12 showed no relationship between the use of immunosuppressants and renal function with KLA, indicating that KLA levels might be affected by non-renal-related factors (24). Our data suggested that vitamin D significantly contributed to the increase in soluble KLA levels within 12 months posttransplant. However, we believe that the effect of vitamin D supplementation on KLA in KTRs requires further investigation. First, the expression of the *KLA* gene is regulated by numerous factors other than vitamin D and PTH. Previous studies have reported that the renin–angiotensin–aldosterone system and other factors promoting *FGF23* expression were associated with the downregulation of *KLA* gene expression (40), while thiazolidinediones could induce the expression of the *KLA* gene (41). Adiponectin and leptin were also reported to be correlated with the increase and reduction in KLA levels in KTRs (42). Secondly, the soluble KLA level is closely related to the ectodomain shedding of membrane KLA. Yoon *et al.* reported a novel pathway consisting of Ca-sensing receptor, ADAM metallopeptidase domain 10 and KLA. The pathway was activated by Ca-sensing receptor activators, including serum calcium, calcimimetics and alkali, and resulted in the increased shedding of membrane KLA and elevated soluble KLA (43). Given that cinacalcet was prescribed to approximately 10% of KTRs in the treated group, the effect of increased ectodomain shedding on elevated soluble KLA levels should not be ignored. Therefore, a randomized, double-blinded controlled trial with larger samples is needed to identify the independent effect of vitamin D supplementation on soluble KLA levels.

Another encouraging result in our study was that the levels of BCTX and P1NP were significantly lower in the treated group than in the untreated group since 6 months posttransplant (Tables 2 and 3). P1NP is a metabolic marker of bone formation, while BCTX is a marker of bone resorption. The increased BCTX and P1NP levels in KTRs since month 3 indicated the high turnover status of bone metabolism, which will induce the increased loss of bone mass. Vitamin D supplementation will increase the active

vitamin D levels *in vivo*, which plays a dual-directional regulatory effect according to the different types of bone metabolic disorders. For the KTRs in our study, vitamin D supplementation suppressed the high turnover status of KTRs in the treated group and may decrease the bone mass loss thereafter.

Regarding graft function, we found higher eGFR levels in the treated group than in the untreated group. One possible explanation is that vitamin D can induce immune tolerance by regulating the effector function of immune cells and suppressing the secretion of proinflammatory cytokines and chemokines (36), so it is possible that graft function can be affected by vitamin D supplementation. However, the effect of vitamin D on graft function remains controversial. A recent randomized controlled trial from Japan showed that vitamin D supplementation exerted no effect on graft function and pathological changes in renal grafts within 12 months posttransplant (22). One possible reason for the inconsistent result between our study and the Japanese study was that the average 25(OH)D level in our treated group was 89.7 ± 16.00 nmol/L at month 12, which was much higher than that in the Japanese study, with a median level of 40 (30–49) nmol/L. However, we found no correlation between vitamin D supplementation and graft function at month 12, which might be associated with limited follow-up time. Thus, the effect of vitamin D supplementation on graft function needs to be evaluated with more immune markers and longer follow-up times.

In our study, we discovered that vitamin D supplementation before and after KT delayed the progression of coronary artery calcification within 12 months posttransplant. Vitamin D deficiency was reported to be associated with coronary artery calcification by previous studies concerning both CKD patients and KTRs. The protective effect of vitamin D on vascular calcification after KT is likely multifactorial. First, excessively elevated levels of PTH and serum calcium were associated with the development and progression of extraskeletal calcification (44, 45). Sufficient vitamin D is able to suppress the secretion of PTH and increase skeletal calcium deposition, thus preventing the development and progression of vascular calcification. Secondly, emerging evidence suggests that the anti-inflammatory effect of vitamin D contributes to preventing endothelial dysfunction and vascular calcification. The expression of several cytokines involved in the development of the early stage of vascular calcification, including IL1, IL6, IL8 and TNF, could be reduced by vitamin D in a paracrine way or an intracrine way in monocytes (46, 47, 48). Thirdly, as an antiaging protein, KLA was also reported to be protective against vascular calcification in both soluble and membrane-bound forms (49, 50). Existing evidence has demonstrated that KLA can suppress the phosphate-induced calcification of vascular smooth cells and reduce the expression of plasminogen activator inhibitor 1, p21, p16 and senescence-associated galactosidase beta, which are involved in cell senescence and lead to damage to endothelial integrity (51). As a noninvasive examination, CACS was reported to be an early independent predictor of cardiovascular morbidity and mortality in haemodialysis patients (52, 53). However, no cardiovascular events were reported in either group of our study. The impact of vitamin D supplementation on the incidence of cardiovascular disease after KT needs to be evaluated with a longer follow-up.

To our knowledge, our study is the first to prospectively explore the effects of vitamin D supplementation on iFGF23 and KLA levels and CACS in KTRs. However, there were still a few limitations. First, our study was a nonrandomized cohort trial, which may induce selection bias. Secondly, the follow-up time in our study was only 12 months, which might not be sufficient to observe the impacts of vitamin D supplementation on bone mineral metabolism, graft function and cardiovascular events. Thirdly, our sample size was not large enough. Therefore, randomized control studies with a longer follow-up time and a larger sample size should be performed to further assess the role of vitamin D supplementation in KTRs.

Conclusion

In summary, THPT and disorders of the FGF23–KLA axis occurred in most KTRs with ordinary graft function within 12 months posttransplant. Coronary calcification also developed with poorly controlled THPT and elevated iFGF23 levels after transplantation. Correction of vitamin D deficiency using cholecalciferol within 12 months posttransplant was effective in attenuating persistent hyperparathyroidism, improving FGF23 resistance and high bone turnover status, preserving graft function and preventing coronary calcification.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-22-0123.

Declaration of interest

All of the authors have read the manuscript and approved its submission. The manuscript has not been published previously and is not being considered for publication in any language elsewhere, either in whole or in part, except as an abstract.

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Author contribution statement

Yun-Ying Shi and Lan-Lan Wang contributed to the conception and designation of the research. Xian-Ding Wang, Tao Lin, Yun-Ying Shi and Ye Tao contributed to the recruitment and follow-up of subjects. Ya-Mei Li, Shu-Meng Hu and Yang-Juan Bai contributed to the conduct of experiment and the analysis of data. Shu-Meng Hu, Yun-Ying Shi and Lan-Lan Wang contribute to the drafting, revising and the final approval of the article.

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