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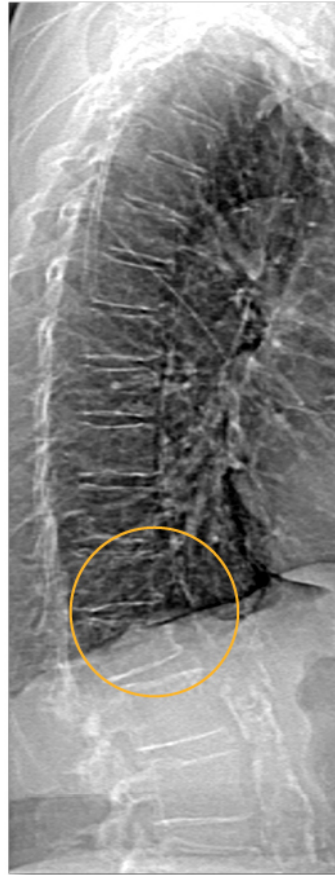
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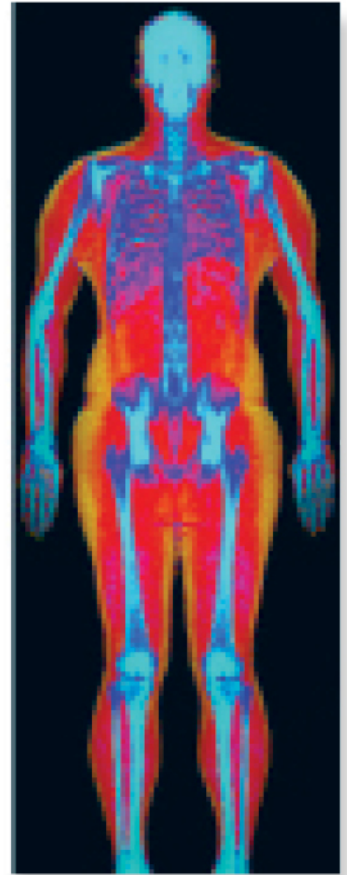
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Cholecalciferol Supplementation Attenuates Bone Loss in Incident Kidney Transplant Recipients: A Prespecified Secondary Endpoint Analysis of a Randomized Controlled Trial

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ABSTRACT

Vitamin D deficiency, persistent hyperparathyroidism, and bone loss are common after kidney transplantation (KTx). However, limited evidence exists regarding the effects of cholecalciferol supplementation on parathyroid hormone (PTH) and bone loss after KTx. In this prespecified secondary endpoint analysis of a randomized controlled trial, we evaluated changes in PTH, bone metabolic markers, and bone mineral density (BMD). At 1 month post-transplant, we randomized 193 patients to an 11-month intervention with cholecalciferol (4000 IU/d) or placebo. The median baseline 25-hydroxyvitamin D (25[OH]D) level was 10 ng/mL and 44% of participants had osteopenia or osteoporosis. At the end of the study, the median 25(OH)D level was increased to 40 ng/mL in the cholecalciferol group and substantially unchanged in the placebo group. Compared with placebo, cholecalciferol significantly reduced whole PTH concentrations (between-group difference of -15% ; 95% confidence interval [CI] -25 to -3), with greater treatment effects in subgroups with lower 25(OH)D, lower serum calcium, or higher estimated glomerular filtration rate ($p_{\text{int}} < 0.05$). The percent change in lumbar spine (LS) BMD from before KTx to 12 months post-transplant was -0.2% (95% CI -1.4 to 0.9) in the cholecalciferol group and -1.9% (95% CI -3.0 to -0.8) in the placebo group, with a significant between-group difference (1.7% ; 95% CI 0.1 to 3.3). The beneficial effect of cholecalciferol on LS BMD was prominent in patients with low bone mass ($p_{\text{int}} < 0.05$). Changes in serum calcium, phosphate, bone metabolic markers, and BMD at the distal radius were not different between groups. In mediation analyses, change in whole PTH levels explained 39% of treatment effects on BMD change. In conclusion, 4000 IU/d cholecalciferol significantly reduced PTH levels and attenuated LS BMD loss after KTx. This regimen has the potential to eliminate vitamin D deficiency and provides beneficial effects on bone health even under glucocorticoid treatment. © 2021 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: KIDNEY TRANSPLANTATION; VITAMIN D; BONE MINERAL DENSITY; PARATHYROID HORMONE; CHRONIC KIDNEY DISEASE-MINERAL AND BONE DISORDER

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Additional Supporting Information may be found in the online version of this article.

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Introduction

Kidney transplantation (KTx) is the treatment of choice for patients with end-stage kidney disease from the viewpoint of cost, quality of life, and mortality.⁽¹⁻³⁾ However, bone mineral density (BMD) declines progressively after KTx⁽⁴⁾ and, accordingly, the fracture risk in kidney transplant recipients (KTRs) is higher than in patients who remain on dialysis in the first 3 years after KTx.⁽⁵⁾ The fracture event in KTRs is associated with higher all-cause mortality.^(6,7)

The bone disease in KTRs is complex and the increased fracture risk after KTx may be explained by several factors, including preexisting mineral bone disorder (MBD), de novo MBD, and immunosuppressive drugs.⁽⁴⁾ Among these factors, secondary hyperparathyroidism and vitamin D deficiency are common and may be modifiable to improve skeletal health among KTRs.⁽⁸⁾ Meta-analyses have demonstrated that vitamin D supplementation effectively restored 25-hydroxyvitamin D (25 [OH]D) levels and reduced circulating parathyroid hormone (PTH) levels in subjects with preserved kidney function and predialysis chronic kidney disease but not in patients on dialysis.⁽⁹⁻¹¹⁾ However, it is not fully investigated whether vitamin D supplementation reduces PTH concentration among KTRs, a population with potentially reduced expression of vitamin D receptor (VDR) in parathyroid glands similar to patients on dialysis.⁽¹²⁾

Controversy exists regarding the efficacy of vitamin D supplementation on BMD.^(13,14) The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines published in 2009 recommended performing randomized controlled trials (RCTs) to confirm the effect of vitamin D supplementation on BMD among KTRs with vitamin D deficiency;⁽¹⁵⁾ however, no RCTs on this issue have been published since then. Therefore, we evaluated the effect of cholecalciferol on MBD parameters, including PTH levels and BMD, in a prespecified secondary endpoint analysis of a randomized, double-blind, placebo-controlled trial among incident KTRs.

Materials and Methods

Study design and participants

This study is a prespecified secondary endpoint analysis of a single-center, parallel-arm, randomized, double-blind, placebo-controlled, 11-month trial reported in detail elsewhere.⁽¹⁶⁾ Briefly, this study was conducted in Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, Japan, among KTRs at 1 month after living KTx who were aged 20 to 80 years and had an estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73 m². Patients taking native vitamin D (ergocalciferol or cholecalciferol) and/or having hypercalcemia, defined as a corrected calcium level of ≥ 11 mg/dL, were excluded. All participants provided written informed consent, and the study adhered to the Declaration of Helsinki. This original trial was approved by the ethics committees of Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital (IRB approval number: 1038) and registered at the UMIN Clinical Trials Registry (ID: UMIN000020597).

Randomization and intervention

Participants were randomized using a computer-generated sequence at an allocation ratio of 1:1 to receive either cholecalciferol 4000 IU or a matching placebo daily from 1 to 12 months

post-transplant, with a random block size of 8 and stratified by age (<50 or ≥ 50 years) and sex. None of the patients received active forms of vitamin D, or antiresorptive or anabolic agents for the prevention or treatment of osteoporosis throughout the study period.

Endpoints

In the present study, we report the following prespecified secondary endpoints: (i) the percent changes in whole PTH, tartrate-resistant acid phosphatase-5b (TRACP-5b), and bone-specific alkaline phosphatase (BAP) levels from baseline to 12 months post-transplant and (ii) the percent changes in BMD at the lumbar spine (LS) and the distal one-third radius from before KTx to 12 months post-transplant.

Measurements

Blood and urine samples were collected at baseline, 6, and 12 months post-transplant and stored at -80°C until assayed. Serum 25(OH)D levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostic GmbH, Mannheim, Germany). Serum 1,25-dihydroxyvitamin D (1,25[OH]₂D) concentrations were evaluated using a radioimmunoassay (Immunodiagnostic Systems Ltd, Boldon, UK). Plasma whole PTH level was measured using a chemiluminescent enzyme immunoassay (Fujirebio, Inc., Tokyo, Japan). Serum TRACP-5b level was evaluated using an enzyme immunoassay (DS Pharma Promo Co., Ltd., Osaka, Japan). Serum BAP level was measured using a one-step immunoenzymatic assay (Beckman Coulter Inc., Brea, CA, USA). BMD measurements were performed at the LS (L₂ through L₄) and the distal one-third radius using dual-energy X-ray absorptiometry (DXA) just before KTx and at 12 months post-transplant using a Hologic Discovery densitometer (Hologic Discovery; Hologic, Inc., Bedford, MA, USA). The eGFR value was calculated on the basis of the Chronic Kidney Disease Epidemiology (CKD-EPI) creatinine-cystatin C equation.⁽¹⁷⁾ Osteoporosis and osteopenia were defined as T-scores of ≤ -2.5 and between -1 and -2.4 , respectively.

Immunosuppressive therapy

Induction immunosuppressive therapies were administered to all participants according to their risk of acute rejection, followed by triple immunosuppression therapies consisting of prednisolone, calcineurin inhibitor (cyclosporine or tacrolimus), and mycophenolate mofetil or everolimus. Corticosteroid therapy was initiated with methylprednisolone 500 mg intravenously on the day of KTx, followed by oral prednisolone 60 mg, with progressive tapering to 5 mg daily by 3 months post-transplant. Then, the prednisolone dosage was maintained at 5 mg daily.

Statistical analysis

Specific sample size calculations were not conducted for prespecified secondary endpoints. The effects of cholecalciferol versus placebo on whole PTH, TRACP-5b, BAP, and BMD levels were estimated by analysis of covariance (ANCOVA), adjusting for baseline values based on the full analysis set that included all randomized participants who took the allocated drugs at least once. Whole PTH, TRACP-5b, and BAP values were log-transformed before analysis and then back-transformed. Post hoc several sensitivity analyses were conducted as follows: (i) using 100 imputed data sets, with missing data handled with

multivariate imputation by chained equations; (ii) using mixed-effects models with repeated measures using all available data and median imputation for missing baseline data; and (iii) excluding patients with poor adherence (defined as taking less than half the allocated drugs during the study). The heterogeneity of treatment effects on whole PTH and LS BMD levels according to baseline characteristics including sex, preemptive kidney transplant, body mass index, eGFR, serum calcium level, phosphate level, 25(OH)D level, and their baseline values was assessed in a post hoc manner. To avoid arbitrary cut-points and preserve the statistical power, we used the multivariable fractional polynomial interaction approach to evaluate the interactions between continuous variables and treatment.⁽¹⁸⁾ Moreover, mediation analyses were performed to evaluate the extent of treatment effects on LS BMD that was explained by the change in whole PTH levels using structural equation modeling. Considering the lack of adjustment for multiplicity, the results should be interpreted with caution. The statistical test was two-tailed, and *p* values <0.05 were considered statistically significant. Analyses were performed with Stata/SE 15 statistical software package (Stata Corp., College Station, TX, USA).

Results

Study patients and study flow

A total of 193 patients were randomized to either cholecalciferol (*n* = 96) or placebo (*n* = 97) (Supplemental Fig. S51). Of these patients, 187 (92 in the cholecalciferol group and 95 in the placebo group) participants who received at least one dose of the study drug were included in the analyses (full analysis set). Baseline characteristics were similar across the two treatment groups, except for higher whole PTH levels in the cholecalciferol group (Table 1). {TBL 1} The median age of the participants was 52 years, and approximately 70% were men. Median eGFR and 25(OH)D levels were 46 mL/min/1.73 m² and 10 ng/mL, respectively. Most participants (96%) had deficient concentrations of serum 25(OH)D (ie, <20 ng/mL).⁽¹⁹⁾ Regarding LS BMD, 44% of them had osteopenia or osteoporosis before KTx. At the end of the study, serum 25(OH)D levels showed minimal changes in the placebo group (3 ng/mL [95% CI 2 to 5]), but substantial increases in the cholecalciferol group (28 ng/mL [95% CI 27 to 30]), resulting in a significant between-group difference of 25 ng/mL (95% CI 22 to 28; Table 2). {TBL 2} There were 77% of patients with a serum 25(OH)D >30 ng/mL at 12 months post-transplant in the cholecalciferol group but none in the placebo group. The increase in serum 1,25(OH)₂D levels in the cholecalciferol group was higher than that in the placebo group (between-group difference 14 pg/mL [95% CI, 9 to 20], Table 2).

Whole PTH, TRACP-5b, BAP, and other laboratory parameters

The geometric mean of the percent change in whole PTH level was -28% (95% CI -34 to -22) in the placebo group and -39% (95% CI -44 to -33) in the cholecalciferol group, with a significant between-group difference (-15% [95% CI -25 to -3, *p* = 0.02]; Table 2 and Fig. 1). {FIG1} The sensitivity analyses also revealed similar results (Supplemental Table S51). The effects of cholecalciferol on whole PTH levels were modified by baseline eGFR, calcium, and 25(OH)D levels, with greater

Table 1. Baseline Characteristics and Laboratory Values

	Cholecalciferol (<i>n</i> = 92)	Placebo (<i>n</i> = 95)
Basic information		
Age (years)	52 (42, 63)	52 (43, 62)
Male sex, <i>n</i> (%)	64 (70)	65 (68)
BMI (kg/m ²)	22.5 (20.4, 24.5)	22.1 (20.3, 24.7)
PEKT, <i>n</i> (%)	45 (49)	52 (55)
Dialysis vintage (months)	2 (0, 17)	0 (0, 13)
ABO compatible transplantation, <i>n</i> (%)	59 (64)	61 (64)
Second transplantation, <i>n</i> (%)	3 (3)	1 (1)
Prior parathyroidectomy, <i>n</i> (%)	0 (0)	2 (2)
Immunosuppressive drugs, <i>n</i> (%)		
Cyclosporine	32 (35)	29 (31)
Tacrolimus	60 (65)	66 (69)
Everolimus	24 (26)	22 (23)
Mycophenolate mofetil	68 (74)	73 (77)
Primary renal disease, <i>n</i> (%)		
Chronic glomerulonephritis	29 (32)	27 (28)
Diabetic nephropathy	24 (26)	27 (28)
Polycystic kidney disease	7 (8)	11 (12)
Hypertensive nephropathy	7 (8)	6 (6)
Others	25 (27)	24 (25)
Laboratory data		
Hemoglobin (g/dL)	11.4 (10.6, 12.2)	11.3 (10.4, 12.0)
Albumin (g/dL)	4.0 (3.8, 4.3)	4.0 (3.8, 4.2)
eGFR (mL/min per 1.73 m ²)	46 (37, 55)	46 (36, 57)
Corrected calcium (mg/dL)	9.5 (9.2, 9.7)	9.4 (9.2, 9.8)
Phosphate (mg/dL)	2.7 (2.1, 3.3)	2.8 (2.1, 3.2)
25(OH)D (ng/mL)	10 (9, 14)	10 (8, 13)
1,25(OH) ₂ D (pg/mL)	46 (30, 59)	42 (34, 51)
Whole PTH (pg/mL)	77 (50, 107)	63 (46, 85)
TRACP-5b (mU/dL)	602 (438, 795)	577 (434, 756)
BAP (μg/L)		
	13 (10, 19)	12 (9, 18)
Bone mineral density <i>T</i> -score		
Distal 1/3 radius	-1.6 (-1.6, -0.8)	-1.4 (-2.5, -0.2)
Lumbar spine	-0.8 (-1.5, 0.0)	-0.9 (-1.8, 0.3)
Normal, <i>n</i> (%)	49 (55)	54 (57)
Osteopenia, ^a <i>n</i> (%)	31 (34)	29 (31)
Osteoporosis, ^b <i>n</i> (%)	9 (10)	12 (13)

BMI = body mass index; PEKT = preemptive kidney transplantation; eGFR = estimated glomerular filtration rate; 25(OH)D = 25-hydroxyvitamin D; 1,25(OH)₂D = 1,25-dihydroxyvitamin D; PTH = parathyroid hormone; TRACP-5b = tartrate-resistant acid phosphatase-5b; BAP = bone-specific alkaline phosphatase.

Data are based on the full analysis set. Continuous variables are presented as median (25th, 75th percentile) or *n* (%).

^aOsteopenia was defined as a *T*-score -1 to -2.5 at the lumbar spine.

^bOsteoporosis was defined as a *T*-score below -2.5 at the lumbar spine.

Table 2. Changes in Biochemical Parameters From Baseline to 12 Months Post-Transplant

Parameter	Treatment	Baseline	6 months	12 months	Within-group difference (95% CI) ^a	<i>p</i> [*]	Between-group difference (95% CI) ^a	<i>p</i> [*]
25(OH)D (ng/mL)	Cholecalciferol	10 (9, 14)	38 (31, 45)	40 (30–49)	28 (26 to 30)	<0.01	25 (22 to 28)	<0.01
	Placebo	10 (8, 13)	13 (10, 17)	14 (10, 18)	3 (2 to 5)	<0.01		
1,25(OH) ₂ D (pg/mL)	Cholecalciferol	46 (30, 59)	68 (47, 89)	69 (50, 62)	21 (17 to 25)	<0.01	14 (9 to 20)	<0.01
	Placebo	42 (34, 51)	47 (39, 64)	51 (43, 62)	7 (3 to 11)	<0.01		
eGFR (mL/min per 1.73 m ²)	Cholecalciferol	46 (37, 55)	45 (36, 53)	46 (37, 57)	1.2 (−0.7 to 3.1)	0.23	−0.6 (−3.3 to 2.0)	0.63
	Placebo	46 (36, 57)	44 (38, 54)	48 (40, 55)	2.8 (−0.01 to 3.7)	0.05		
Corrected calcium (mg/dL)	Cholecalciferol	9.5 (9.2, 9.7)	9.6 (9.3, 9.9)	9.6 (9.3, 9.9)	0.2 (0.03 to 0.3)	0.01	0.1 (−0.1 to 0.2)	0.33
	Placebo	9.4 (9.2, 9.8)	9.5 (9.2, 9.7)	9.5 (9.2, 9.8)	0.1 (−0.04 to 0.2)	0.23		
Phosphate (mg/dL)	Cholecalciferol	2.7 (2.1, 3.3)	3.2 (2.9, 3.5)	3.1 (2.8, 3.4)	0.4 (0.2 to 0.5)	<0.01	0.03 (−0.1 to 0.2)	0.63
	Placebo	2.8 (2.1, 3.2)	3.2 (2.9, 3.5)	3.0 (2.8, 3.5)	0.3 (0.2 to 0.4)	<0.01		
Whole PTH (pg/mL)	Cholecalciferol	77 (50, 107)	42 (30, 60)	40 (31, 56)	−39% (−44 to −33)	<0.01	−15% (−25 to −3) ^b	0.02
	Placebo	63 (46, 85)	47 (34, 64)	47 (33, 61)	−28% (−34 to −22)	<0.01		
TRACP-5b (mU/dL)	Cholecalciferol	602 (438, 795)	344 (252, 502)	315 (236, 429)	−44% (−49 to −38)	<0.01	−2% (−14 to 12) ^b	0.76
	Placebo	577 (434, 756)	366 (263, 497)	308 (232, 443)	−43% (−48 to −37)	<0.01		
BAP (μg/L)	Cholecalciferol	13 (10, 19)	13 (10, 19)	11 (9, 15)	−11% (−19 to −2)	0.02	−8% (−18 to 4) ^b	0.19
	Placebo	12 (9, 18)	14 (10, 18)	13 (9, 16)	−3% (−11 to 6)	0.51		

CI = confidence interval; 25(OH)D = 25-hydroxyvitamin D; 1,25(OH)₂D = 1,25-dihydroxyvitamin D; eGFR = estimated glomerular filtration rate; PTH = parathyroid hormone; TRACP-5b = tartrate-resistant acid phosphatase-5b; BAP = bone-specific alkaline phosphatase.

Data are presented as median (25th, 75th percentile).

^{*}The *p* values were derived from analysis of covariance.

^aAdjustment for baseline values.

^bEstimated values are presented as the geometric mean of the percent change.

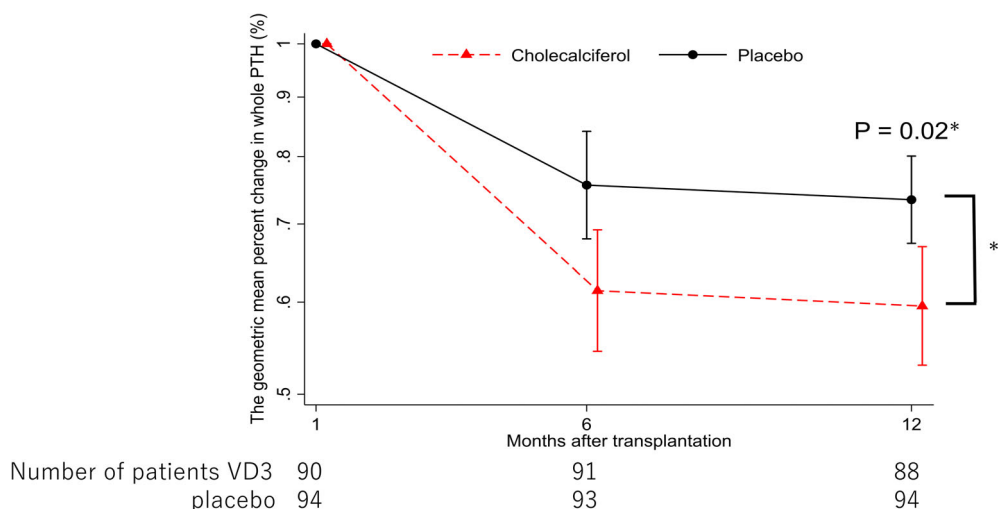


Fig 1. The geometric mean of the percent change in whole parathyroid hormone (PTH) level according to time and trial group. The I bars indicate 95% confidence intervals.

treatment effects being observed in participants with higher eGFR, lower calcium level, and lower 25(OH)D level (all *p* value for interaction <0.05; Fig. 2 and {FIG2} Supplemental Table S2).

Changes in serum TRACP-5b, BAP, calcium, and phosphate concentrations were similar between groups (Table 2 and Supplemental Table SS1).

Bone mineral density

The mean percent change in LS BMD was -0.2% (95% CI -1.4 to 0.9) in the cholecalciferol group and -1.9% (95% CI -3.0 to -0.8) in the placebo group, with a significant between-group difference (1.7% [95% CI 0.1 to 3.3 , $p = 0.04$]; Table 3 and Fig. 3A). Several sensitivity analyses provided robust results (Supplemental Table S51). Adjusting for cumulative corticosteroid doses at 12 months post-transplant (mean doses [prednisolone equivalent] were 2981 mg in the cholecalciferol group and 2920 mg in the placebo group, respectively) did not alter the primary results substantially (data not shown). The effects of cholecalciferol were generally consistent except for baseline BMD (Fig. 4 and Supplemental

Table S2). The beneficial effect of cholecalciferol on LS BMD was prominent in patients with lower baseline BMD at the LS (p for interaction <0.05 ; Fig. 4). For easy interpretation, we conducted stratified analyses according to baseline LS BMD (osteoporosis/osteopenia or not). Results showed that cholecalciferol significantly increased LS BMD in patients with osteoporosis/osteopenia but not in those with normal BMD (between-group differences 3.5% [95% CI 0.9 to 6.1 , $p = 0.01$] and 0.7% [95% CI -1.4 to 2.7 , $p = 0.53$], respectively; Fig. 3B). The percent change in BMD at the distal one-third radius did not differ according to treatment allocation (between-group difference -0.4% [95% CI -1.7 to 0.9 , $p = 0.54$]; Table 2 and Supplemental Table S51).

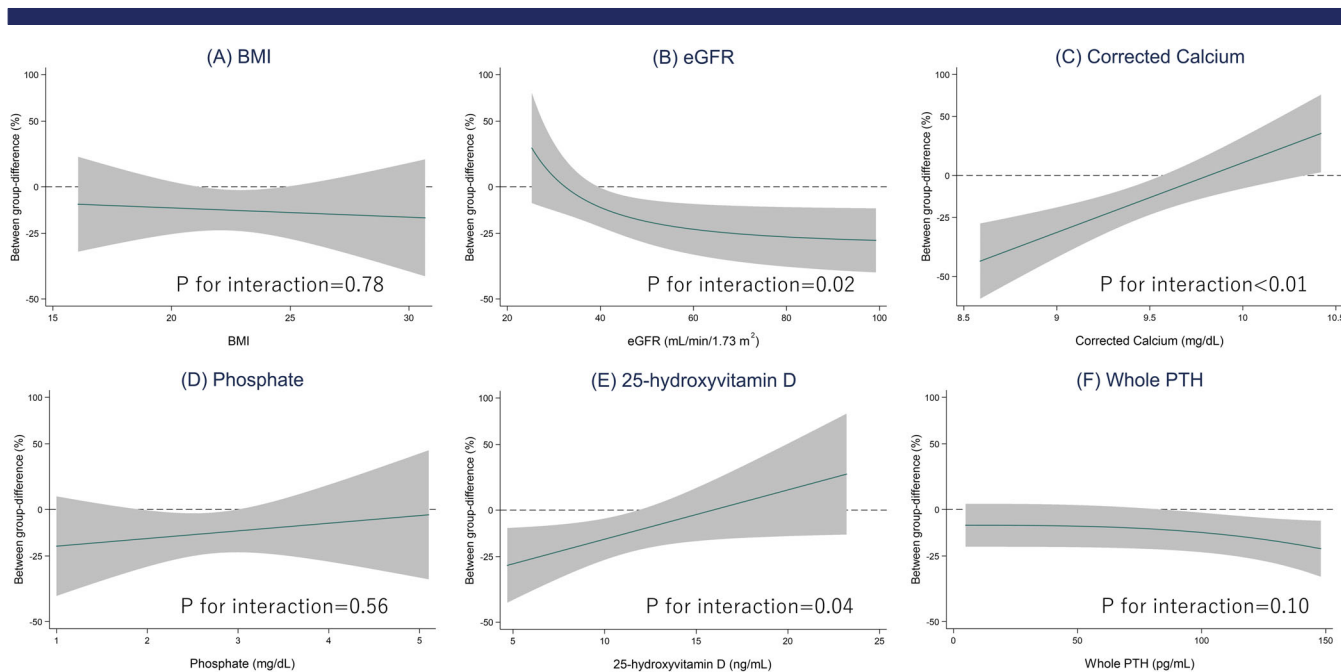


Fig 2. The heterogeneity of treatment effects on changes in whole parathyroid hormone (PTH) level at 12 months post-transplant across baseline (A) body mass index (BMI), (B) estimated glomerular filtration rate (eGFR), (C) corrected calcium, (D) phosphate, (E) 25-hydroxyvitamin D, and (F) whole PTH levels. A negative value on the vertical axis indicates that cholecalciferol reduced whole PTH levels compared with placebo. The p values are for interaction between baseline variables and treatment effect. The green line represents the point of estimate, and the gray area represents the 95% confidence interval.

Table 3. The Mean Percent Changes in Bone Mineral Density From Before Kidney Transplantation to 12 Months Post-Transplant

Treatment	Before KTx	12 months	Within-group difference (95% CI) ^a	p^*	Between-group difference (95% CI) ^a	p^*
Lumbar spine (g/cm^2)						
Cholecalciferol	0.94 (0.87, 1.04)	0.93 (0.85, 1.02)	-0.2% (-1.4 to 0.9) ^b	0.68	1.7% (0.1 to 3.3) ^b	0.04
Placebo	0.94 (0.85, 1.06)	0.92 (0.81, 1.03)	-1.9% (-3.0 to -0.8) ^b	<0.01		
Distal 1/3 radius (g/cm^2)						
Cholecalciferol	0.68 (0.61, 0.76)	0.68 (0.62, 0.76)	-0.8% (-1.7 to 0.1) ^b	0.10	-0.4% (-1.7 to 0.9) ^b	0.54
Placebo	0.72 (0.64, 0.78)	0.71 (0.62, 0.77)	-0.4% (-1.2 to 0.5) ^b	0.39		

KTx = kidney transplantation; CI = confidence interval.

Data are presented as median (25th, 75th percentile).

*The p values were derived from analysis of covariance.

^aAdjustment for baseline values.

^bEstimated values are presented as the mean percent change.

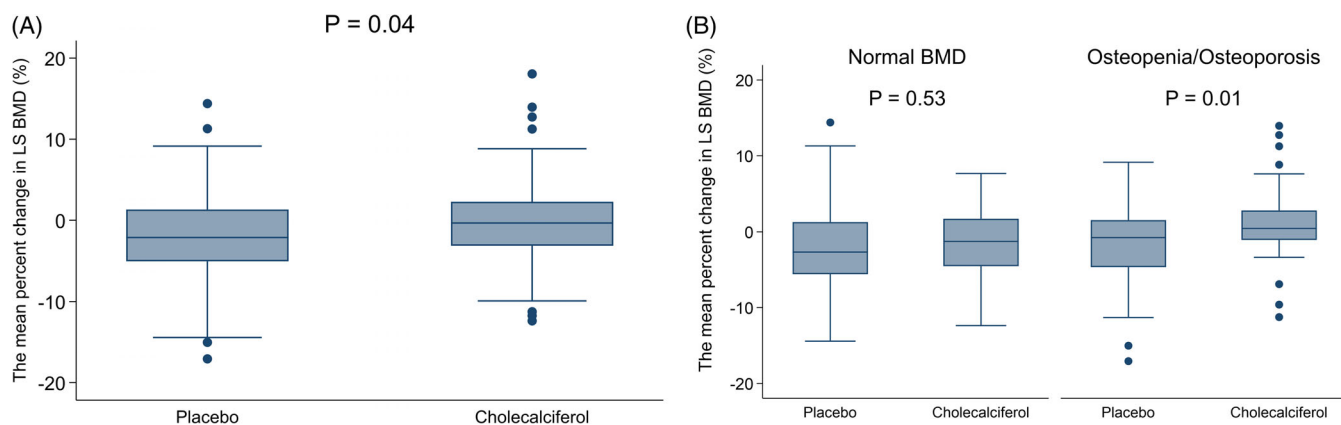


Fig 3. The mean percent change in BMD at the lumbar spine from before kidney transplantation to 12 months post-transplant in the (A) whole population and (B) subgroups with or without osteopenia/osteoporosis. Box plots show median, interquartile, $1.5 \times$ interquartile range, and outliers. BMD = bone mineral density.

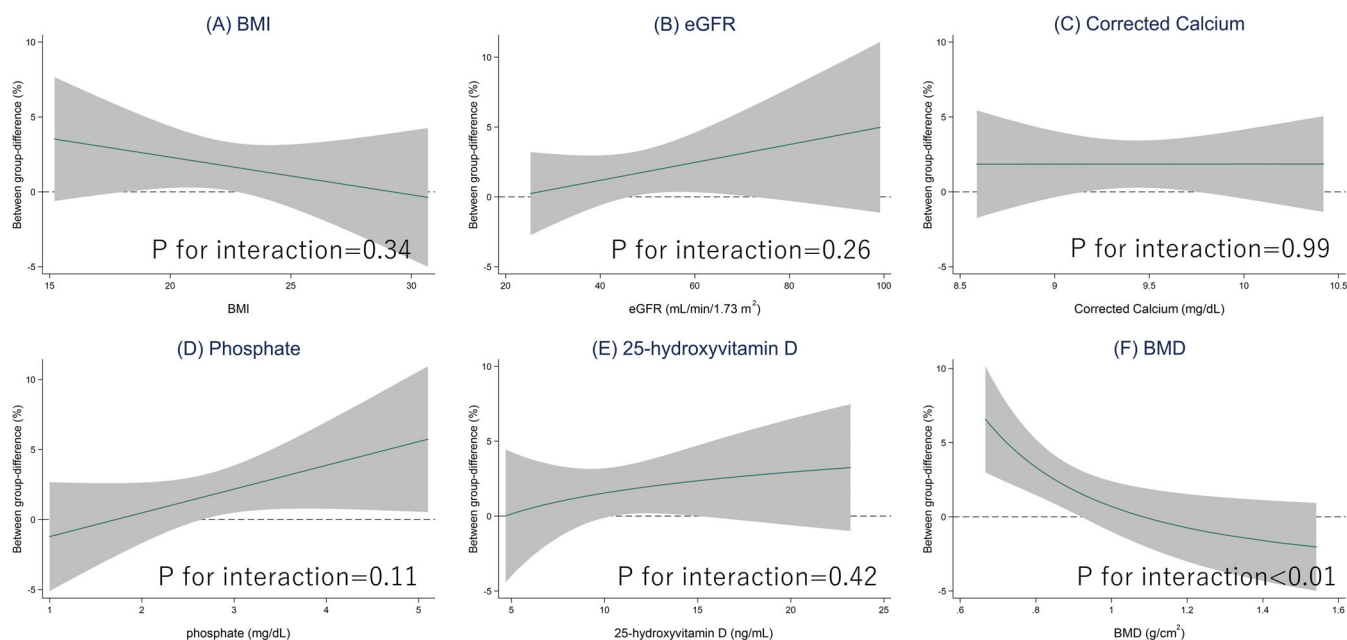


Fig 4. The heterogeneity of treatment effects on changes in lumbar spine (LS) bone mineral density (BMD) at 12 months post-transplant across baseline (A) body mass index (BMI), (B) estimated glomerular filtration rate (eGFR), (C) corrected calcium, (D) phosphate, (E) 25-hydroxyvitamin D, and (F) LS BMD levels. A positive value on the vertical axis indicates that cholecalciferol increased LS BMD compared with placebo. The p values are for interaction between baseline variables and treatment effect. The green line represents the point of estimate, and the gray area represents the 95% confidence interval.

Mediating effect of change in whole PTH on LS BMD

As shown in Table 2, cholecalciferol significantly reduced whole PTH levels compared with placebo. Furthermore, changes in PTH levels from baseline to 12 months post-transplant were associated with changes in LS BMD ($p < 0.01$). In the mediation analysis using structural equation modeling, the indirect effect mediated by changes in whole PTH levels was 39% ($p = 0.02$).

Adverse effects

During the study period, the incidences of hypercalcemia, all-cause death, and fracture were similar between groups (Supplemental Table S3). Three fractures were identified, *vis.*, distal radius fracture and patellar fracture in the cholecalciferol group and scaphoid fracture in the placebo group.

Discussion

In this prespecified analysis of a randomized, double-blind, placebo-controlled trial among incident KTRs, we observed that cholecalciferol significantly reduced whole PTH levels and attenuated bone loss at the LS compared with placebo. The treatment effects of cholecalciferol on whole PTH level were greater among participants with high eGFR, low calcium level, and low 25(OH)D level. Meanwhile, its effects on LS BMD were prominent in patients with low BMD (eg, osteopenia/osteoporosis).

The key finding of the present study was that cholecalciferol decreased whole PTH levels compared with placebo. Previous studies have suggested that, with a decline in kidney function, the effect of vitamin D supplementation on PTH secretion diminished because of a progressive loss of VDR in the parathyroid gland.^(20,21) Consistently, most RCTs conducted among patients on dialysis failed to demonstrate a beneficial effect of vitamin D supplementation on PTH levels.^(9,10,22-24) Meanwhile, Taniguchi and colleagues demonstrated that successful KTx causes regression of the parathyroid gland with diffuse hyperplasia and restores its expression of VDR.⁽¹²⁾ Probable VDR restoration and high 25(OH)D levels, coupled with accelerated conversion of 25(OH)D to 1,25(OH)₂D due to secondary hyperparathyroidism and healthy kidney transplanted, could contribute to PTH reduction by cholecalciferol supplementation in the current study. Besides, greater expression of CYP27b1 in parathyroid cells among patients with secondary hyperparathyroidism may strengthen its PTH-lowering effects.^(25,26) We observed the heterogeneity in terms of treatment effects on PTH changes according to baseline serum 25(OH)D, calcium, and eGFR levels. Based on this observation, the relative contribution of vitamin D to suppress PTH levels should be minor in the setting of hypercalcemia because high serum calcium level, per se, suppresses PTH secretion through the calcium-sensing receptor on the parathyroid gland. Regarding a diminished treatment effect in patients with impaired renal function, we speculated that insufficient improvement of uremia could hinder the restoration of VDR in the parathyroid hyperplasia among KTRs.⁽¹²⁾

To date, the only small open-label RCT of 79 incident KTRs demonstrated that a monthly single dose of 25,000 IU cholecalciferol with oral calcium significantly reduced intact PTH levels compared with oral calcium alone.⁽²⁷⁾ We confirmed this result based on a larger, double-blind, placebo-controlled trial. Hence, cholecalciferol supplementation can be a promising strategy to reduce PTH levels among KTRs without increasing serum calcium levels. Considering the relationship between high PTH levels and worse outcomes, including incident fracture, graft survival, and all-cause mortality among KTRs,⁽²⁸⁻³⁰⁾ further studies are required to determine whether vitamin D supplementation provides benefits on long-term outcomes.

An earlier study demonstrated that a decrease in LS BMD was 6.8% during the first 6 months post-transplant, with a further decline in subsequent years.⁽³¹⁾ In contrast, consistent with our data, recent studies have reported that the BMD loss in the first year post-transplant is limited probably because of less cumulative glucocorticoid exposure.⁽³²⁻³⁵⁾ We observed that cholecalciferol supplementation completely canceled the BMD loss observed in the placebo group under low-dose glucocorticoid treatment (a maintenance dose of 5 mg prednisolone). To date, only one randomized trial has been conducted to evaluate the effect of cholecalciferol supplementation on bone loss among KTRs, which reported a rather detrimental effect on BMD loss,

although not statistically significant.⁽²⁷⁾ This discrepancy with our data may be explained by differences in the administration doses and/or methods (4000 IU daily versus a single monthly dose of 25,000 IU). The bolus administration of high-dose vitamin D has been reported to be associated with an acute increase in bone resorption markers independent of PTH levels.⁽³⁶⁾ The small changes in LS BMD by cholecalciferol compared with bisphosphonate⁽³³⁾ raise suspicion with clinical significance for fracture prevention. However, because favorable bisphosphonate response is attained under optimal vitamin D status,⁽³⁷⁾ we think that cholecalciferol is a safe, effective, and inexpensive foundation for preventing bone loss among incident KTRs.

In the mediation analysis, the change in whole PTH levels explained 40% of treatment effects on LS BMD. However, caution is necessary when interpreting this finding because unmeasured mediator-outcome confounding (eg, calcium intake) may influence this result. Moreover, mediation analyses do not guarantee causality. A possible explanation for the positive bone effect of cholecalciferol could be the treatment of undetected mineralization abnormality, which is common among KTRs.^(33,38) Some studies have reported that mineralization abnormality occurred or worsened after KTx,^(31,39) probably through hypophosphatemia in the early phase of the post-transplant period. Additionally, a previous histomorphometric analysis demonstrated that a 25(OH)D level of <30 ng/mL was associated with bone mineralization defects.⁽⁴⁰⁾ Further studies are required on the histomorphometric evaluation to clarify the mechanisms underlying the effect of cholecalciferol supplementation on LS BMD.

The present study had several limitations. It was a single-center study involving only Japanese KTRs from living donors, thereby limiting generalizability to wider populations. Most participants had poor vitamin D status at baseline; hence, these results may not extrapolate to sufficient vitamin D KTRs. The lack of inclusion/exclusion criteria for BMD allowed us to enroll individuals with normal BMD, who comprised more than half of the total population, thereby diluting the effect size on BMD in KTRs at high risk for fracture. A lack of bone histomorphometric evaluation prohibited us from detecting bone mineralization abnormality. The strength of this study was the double-blind, placebo-controlled design. The high proportion of participants who completed the study (96%) was another strength.

In summary, compared with placebo, cholecalciferol supplementation resulted in a greater decrease in whole PTH levels and a stabilization in BMD at the LS among incident KTRs. Although the recently updated KDIGO guidelines recommend correcting low 25(OH)D levels in KTRs, a specific protocol has not been proposed.⁽⁴¹⁾ Cholecalciferol supplementation at 4000 IU daily could be a promising regimen for KTRs with vitamin D deficiency. Further studies are required to evaluate the protective effects of cholecalciferol not only on surrogate markers but also on clinical outcomes including parathyroidectomy and fracture.

Disclosures

All authors state that they have no conflicts of interest.

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Peer Review

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, T.H, upon reasonable request.

References

- Laupacis A, Keown P, Pus N, et al. A study of the quality of life and cost-utility of renal transplantation. *Kidney Int.* 1996;50(1):235-242.
- Russell JD, Beecroft ML, Ludwin D, Churchill DN. The quality of life in renal transplantation: a prospective study. *Transplantation.* 1992;54(4):656-660.
- Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med.* 1999;341(23):1725-1730.
- Bouquegneau A, Salam S, Delanaye P, Eastell R, Khwaja A. Bone disease after kidney transplantation. *Clin J Am Soc Nephrol.* 2016;11(7):1282-1296.
- Ball AM, Gillen DL, Sherrard D, et al. Risk of hip fracture among dialysis and renal transplant recipients. *JAMA.* 2002;288(23):3014-3018.
- Ferro CJ, Arnold J, Bagnall D, Ray D, Sharif A. Fracture risk and mortality post-kidney transplantation. *Clin Transplant.* 2015;29(11):1004-1012.
- Iseri K, Carrero JJ, Evans M, et al. Fractures after kidney transplantation: incidence, predictors, and association with mortality. *Bone.* 2020;140:115554.
- Messa P, Regalia A, Alfieri CM. Nutritional vitamin D in renal transplant patients: speculations and reality. *Nutrients.* 2017;9(6):550.
- Con. nutritional vitamin D replacement in chronic kidney disease and end-stage renal disease. *Nephrol Dial Transplant.* 2020;36(3):566-567.
- Agarwal R, Georgianos PI. Con: nutritional vitamin D replacement in chronic kidney disease and end-stage renal disease. *Nephrol Dial Transplant.* 2016;31(5):706-713.
- Swart KM, Lips P, Brouwer IA, et al. Effects of vitamin D supplementation on markers for cardiovascular disease and type 2 diabetes: an individual participant data meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2018;107(6):1043-1053.
- Taniguchi M, Tokumoto M, Matsuo D, et al. Persistent hyperparathyroidism in renal allograft recipients: vitamin D receptor, calcium-sensing receptor, and apoptosis. *Kidney Int.* 2006;70(2):363-370.
- Bolland MJ, Grey A, Avenell A. Effects of vitamin D supplementation on musculoskeletal health: a systematic review, meta-analysis, and trial sequential analysis. *Lancet Diab Endocrinol.* 2018;6(11):847-858.
- Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *Lancet.* 2014;383(9912):146-155.

- Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int Suppl.* 2009;113:S1-S130.
- Doi Y, Tsujita M, Hamano T, et al. The effect of cholecalciferol supplementation on allograft function in incident kidney transplant recipients: a randomized controlled study. *Am J Transplant.* 2021;21(9):3043-3054.
- Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* 2012;367(1):20-29.
- Royston P, Sauerbrei W. Two techniques for investigating interactions between treatment and continuous covariates in clinical trials. *Stata J.* 2009;9(2):230-251.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96(7):1911-1930.
- Zisman AL, Hristova M, Ho LT, Sprague SM. Impact of ergocalciferol treatment of vitamin D deficiency on serum parathyroid hormone concentrations in chronic kidney disease. *Am J Nephrol.* 2007;27(1):36-43.
- Marckmann P, Agerskov H, Thinesh Kumar S, et al. Randomized controlled trial of cholecalciferol supplementation in chronic kidney disease patients with hypovitaminosis D. *Nephrol Dial Transplant.* 2012;27(9):3523-3531.
- Singer R, Chacko B, Talaulikar G, Karpe K, Walters G. Placebo-controlled, randomized clinical trial of high-dose cholecalciferol in renal dialysis patients: effect on muscle strength and quality of life. *Clin Kidney J.* 2019;12(2):281-287.
- Obi Y, Yamaguchi S, Hamano T, et al. Effect of cholecalciferol on serum hepcidin and parameters of anaemia and CKD-MBD among haemodialysis patients: a randomized clinical trial. *Sci Rep.* 2020;10(1):15500.
- Xu C, Li YC, Zhao SM, Li ZX. Evaluation of responses to vitamin D3 (cholecalciferol) in patients on dialysis: a systematic review and meta-analysis. *J Investig Med.* 2016;64(5):1050-1059.
- Correa P, Segersten U, Hellman P, Akerstrom G, Westin G. Increased 25-hydroxyvitamin D3 1 α -hydroxylase and reduced 25-hydroxyvitamin D3 24-hydroxylase expression in parathyroid tumors: new prospects for treatment of hyperparathyroidism with vitamin D. *J Clin Endocrinol Metab.* 2002;87(12):5826-5829.
- Segersten U, Correa P, Hewison M, et al. 25-hydroxyvitamin D(3)-1 α -hydroxylase expression in normal and pathological parathyroid glands. *J Clin Endocrinol Metab.* 2002;87(6):2967-2972.
- Wissing KM, Broeders N, Moreno-Reyes R, Gervy C, Stallenberg B, Abramowicz D. A controlled study of vitamin D3 to prevent bone loss in renal-transplant patients receiving low doses of steroids. *Transplantation.* 2005;79(1):108-115.
- Perrin P, Caillard S, Javier RM, et al. Persistent hyperparathyroidism is a major risk factor for fractures in the five years after kidney transplantation. *Am J Transplant.* 2013;13(10):2653-2663.
- Doi Y, Hamano T, Ichimaru N, et al. Serum phosphate levels modify the impact of parathyroid hormone levels on renal outcomes in kidney transplant recipients. *Sci Rep.* 2020;10(1):13766.
- Pihlström H, Dahle DO, Mjøen G, et al. Increased risk of all-cause mortality and renal graft loss in stable renal transplant recipients with hyperparathyroidism. *Transplantation.* 2015;99(2):351-359.
- Julian BA, Laskow DA, Dubovsky J, Dubovsky EV, Curtis JJ, Quarles LD. Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med.* 1991;325(8):544-550.
- Evenepoel P, Claes K, Meijers B, et al. Natural history of mineral metabolism, bone turnover and bone mineral density in de novo renal transplant recipients treated with a steroid minimization immunosuppressive protocol. *Nephrol Dial Transplant.* 2020;35(4):697-705.
- Marques IDB, Araújo M, Gracioli FG, et al. A randomized trial of Zoledronic acid to prevent bone loss in the first year after kidney transplantation. *J Am Soc Nephrol.* 2019;30(2):355-365.
- Smerud KT, Dolgos S, Olsen IC, et al. A 1-year randomized, double-blind, placebo-controlled study of intravenous ibandronate on bone

- loss following renal transplantation. *Am J Transplant.* 2012;12(12):3316-3325.
35. Bonani M, Frey D, Brockmann J, et al. Effect of twice-yearly Denosumab on prevention of bone mineral density loss in de novo kidney transplant recipients: a randomized controlled trial. *Am J Transplant.* 2016;16(6):1882-1891.
 36. Rossini M, Gatti D, Viapiana O, et al. Short-term effects on bone turnover markers of a single high dose of oral vitamin D3. *J Clin Endocrinol Metabol.* 2012;97(4):E622-E626.
 37. Carmel AS, Shieh A, Bang H, Bockman RS. The 25(OH)D level needed to maintain a favorable bisphosphonate response is ≥ 33 ng/ml. *Osteoporos Int.* 2012;23(10):2479-2487.
 38. Monier-Faugere MC, Mawad H, Qi Q, Friedler RM, Malluche HH. High prevalence of low bone turnover and occurrence of osteomalacia after kidney transplantation. *J Am Soc Nephrol.* 2000;11(6):1093-1099.
 39. Keronen S, Martola L, Finne P, Burton IS, Kröger H, Honkanen E. Changes in bone histomorphometry after kidney transplantation. *Clin J Am Soc Nephrol.* 2019;14(6):894-903.
 40. Priemel M, von Domarus C, Klatter TO, et al. Bone mineralization defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. *J Bone Miner Res.* 2010;25(2):305-312.
 41. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO. 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int Suppl.* 2017;7(1):1-59.