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Prevalence and correlates of 25-hydroxyvitamin D deficiency in the Chronic Kidney Disease in Children (CKiD) cohort

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Abstract

Background—Vitamin D plays an important role in the mineral and bone disorder seen in chronic kidney disease (CKD). Deficiency of 25-hydroxyvitamin D (25OHD) is highly prevalent in the adult CKD population.

Methods—The prevalence and determinants of 25OHD deficiency (defined as a level <20 ng/ml) were examined longitudinally in 506 children in the CKiD cohort. Predictors of secondary hyperparathyroidism and the determinants of 1,25-dihydroxyvitamin D (1,25(OH)₂D) levels were also evaluated.

Results—Deficiency of 25OHD was observed in 28 % of the cohort at enrollment. Significant predictors of 25OHD deficiency were older age, non-white race, higher body mass index, assessment during winter, less often than daily milk intake, non-use of nutritional vitamin D

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Ethical approval The CKiD study design and conduct were approved by an external advisory committee appointed by the National Institutes of Health and by the Institutional Review Boards at each participating center.

supplement and proteinuria. Lower values of glomerular filtration rate (GFR), serum 25OHD, calcium and higher levels of FGF23 were significant determinants of secondary hyperparathyroidism. Lower GFR, low serum 25OHD, nephrotic-range proteinuria, and high FGF23 levels were significant determinants of serum 1, 25(OH)₂D levels.

Conclusions—Deficiency of 25OHD is prevalent in children with CKD and is associated with potentially modifiable risk factors such as milk intake, nutritional vitamin D supplement use, and proteinuria. 25OHD deficiency is a risk factor for secondary hyperparathyroidism and decreased serum 1, 25(OH)₂D in children with CKD.

Keywords

Mineral metabolism; Pediatric; Chronic renal disease; Hyperparathyroidism; Fibroblast growth factor 23

Introduction

Chronic kidney disease (CKD) carries a high morbidity in children and adults [1–3]. Vitamin D is thought to play an important role in various disease processes that are closely associated with CKD, such as mineral bone disorder, anemia, inflammation, infection, elevated blood pressure, and proteinuria [4–7]. Vitamin D regulates multiple signaling pathways that are linked to renal injury [5, 8] and in adults with CKD, treatment with vitamin D analogs is associated with reduction in proteinuria and inflammation and improved survival [9, 10].

Deficiency of 25OHD is highly prevalent in the general pediatric population [11, 12]. Data from the National Health and Nutrition Examination Survey (NHANES) 2001–2004 showed that 25OHD levels were below 20 ng/ml in 29 % of children [11]. In those children, 25OHD deficiency was associated with older age, female gender, non-white race, higher body mass index (BMI), more time spent watching a television or computer screen, and low intake of milk and vitamin D supplements [11]. Individuals with CKD are exposed to additional risk factors for 25OHD deficiency such as restricted intake of dairy products, inadequate sunlight exposure, impaired photoconversion of vitamin D due to uremia [13] and urinary losses of vitamin D due to heavy proteinuria [14]. However, it is not clear if such CKD-specific risk factors actually result in a higher prevalence of 25OHD deficiency in the CKD population. Some studies of adults with CKD report a higher prevalence of deficiency in the later stages of CKD [15, 16] whereas others find no association with glomerular filtration rate (GFR) [17, 18]. An inverse association of vitamin D levels with proteinuria has been reported in the adult CKD population [19, 20]. Studies of children with CKD published to date are mostly cross-sectional and report a prevalence of 25OHD deficiency that ranges from 30 to 60 % [21–24]. The reported association between 25OHD levels and GFR is variable, with some studies reporting deficiency at lower GFRs [25] and others finding no such association [26]. Two previous studies in children with nephrotic syndrome and normal GFR have evaluated the effect of proteinuria on 25OHD levels. Lower 25OHD levels were found even during remission when compared to healthy controls in one cross-sectional study [27] and significantly lower levels of 25OHD were found during periods of relapse when compared to periods of remission in a study that followed children with nephrotic syndrome longitudinally [28]. No study of children with mild to moderate CKD has evaluated the

association between 25OHD deficiency and proteinuria, and few have assessed the impact of dietary factors and vitamin D supplement use.

The objectives of the present study were to determine the prevalence of and longitudinal changes in 25OHD deficiency and to evaluate demographic, dietary, and CKD-specific risk factors for 25OHD deficiency in children with mild to moderate CKD. Determinants of secondary hyperparathyroidism and 1,25(OH)₂D levels were also evaluated.

Methods

Study population

The Chronic Kidney Disease in Children (CKiD) study, a multicenter, prospective cohort study, enrolled 586 children from 48 pediatric nephrology centers in North America. Eligible participants were between the ages of 1 and 16 years with an estimated GFR between 30 and 90 ml/min/1.73 m², determined by the original Schwartz equation [29]. The CKiD study design and conduct were approved by an external advisory committee appointed by the National Institutes of Health and by the Institutional Review Boards at each participating center. The study design and objectives have been published [30]. As per study design, the serum concentrations of 25OHD, 1,25(OH)₂D, parathyroid hormone (PTH), and plasma fibroblast growth factor 23 (FGF23) were measured at study visits that occurred 3–6 months after enrollment and at 2, 4, and 6 years after the enrollment visit. These visits occurred between June 2005 and March 2013.

Laboratory assays

Serum concentrations of 25OHD were measured in duplicate by chemiluminescence immunoassay (DiaSorin LIAISON 25 (OH)D TOTAL Assay) [31, 32]; inter- and intra-assay coefficients of variation (CVs) were 11.2 and 8.1 %, respectively. 1, 25(OH)₂D was measured by radioimmunoassay [33]; CVs were 12.6 and 9.8 %, respectively. Plasma C-terminal FGF23 concentrations were measured in duplicate by second-generation ELISA (Immutopics Int., San Clemente, CA, USA). GFR was determined by directly measured plasma Iohexol (GE Healthcare, Amersham Division, Princeton, NJ, USA) disappearance curves at enrollment, 1 year later, and every other year thereafter; as described [34]. When not directly measured, GFR was estimated by the CKiD estimating equation using serum creatinine and cystatin C concentrations [35]. Serum creatinine was measured using an enzymatic method on the Bayer Advia 2400 analyzer (Siemens Diagnostics, Tarrytown, NY, USA). Intact PTH was measured by chemiluminometric assays (from June 2006 to March 2010, Advia Centaur System, Bayer Healthcare LLC, reference range 14–72 pg/ml; from April 2010 onwards, Roche e601, Roche Diagnostics, Indianapolis, IN, reference range 15–65 pg/ml). Calcium was measured using the arsenazo dye end-point method and phosphorus was measured using the phosphomolybdate reaction. Serum calcium concentrations were corrected for serum albumin levels: corrected calcium=measured calcium+0.8 × (4.0 serum albumin). Urine protein and creatinine concentrations were determined on first-morning specimens by a Beckman Coulter AU400 autoanalyzer (Beckman Coulter, Inc., Brea, CA, USA). As serum phosphorus concentrations vary with age in healthy children, we expressed

the phosphorus value for each participant as a z-score relative to age-matched values in 493 healthy children 1–20 years old [36].

Other covariates

Demographic data were collected from questionnaires administered during the enrollment visit. Race and ethnicity were self-reported. Maternal education status was reported as high school, some college, and college or more. Dietary data was obtained from a validated food-frequency questionnaire administered at each visit that asked about consumption of various food items in the past 30 days [37]. Screen time was calculated from the reported number of hours spent watching television, playing video games, or using a computer on an average school day. Nutritional and active vitamin D use in the past 30 days were ascertained using the medication and supplement inventory, and use was defined as either a yes or no, as there was a paucity of data regarding dose administered. Latitude of residence and season of blood draw (winter: November–March; summer: April–October) were used to assess sunlight exposure. BMI z-scores were calculated using the Centers for Disease Control and Prevention (2000) Clinical Growth Charts [38].

Statistical analysis

Demographic and clinical characteristics of the study population were summarized using median and interquartile range (IQR) for continuous variables and percentages and frequencies for categorical variables. Participant characteristics at baseline were summarized for the cohort and by 25OHD categories (<20 ng/ml and ≥20 ng/ml). Statistical differences between these categories were determined using the Wilcoxon rank sum test or the Chi-square test as appropriate. The primary outcome was 25OHD deficiency, defined as a serum concentration less than 20 ng/ml [39]. Predictors of 25OHD deficiency were analyzed using a mixed-effects logistic regression model that included a random subject effect to account for repeated measurements of the outcome within each subject. Variables were included in the model if they were expected to have an effect on vitamin D levels or if the *p* values in univariate analysis were <0.1. Covariates evaluated were age, sex, race, maternal education (> high school vs. high school), screen time (<3 h, 3–4 h, or ≥5 h), BMI z-scores, season of blood draw, duration of follow-up, milk intake (daily vs. less often than daily), nutritional and active vitamin D supplementation (yes vs. no), GFR, urinary protein to creatinine (Up/c) ratio (categorized as <0.5, 0.5–1.99, and ≥2), and plasma FGF23 (categorized as tertiles: <102, 102–174, >174 IU/ml). Covariates expected to have a delayed effect on 25OHD (BMI, screen time, ieGFR, and Up/c) were taken from the prior visit (lagged by 1 year). Covariates expected to have an immediate effect on 25OHD (vitamin D supplementation, season of blood draw, and milk intake) were taken from the concurrent visit. Proteinuria was examined in two separate models, as a concurrent covariate and as a lagged covariate, as it can effect 25OHD levels both immediately and long term. Latitude was not a significant predictor of 25OHD deficiency and was excluded from the final model.

Secondary hyperparathyroidism was defined as intact parathyroid hormone (iPTH) levels ≥65 pg/ml, and its predictors were determined using a mixed-effects logistic regression analysis. Predictors of 1,25(OH)₂D levels were determined using a mixed-effects linear

regression analysis. All analyses were conducted using STATA 13 (Stata Corp, College Station, TX, USA).

Results

Cohort characteristics

The study cohort consisted of 506 children with 1016 person-visits over 6 years; 35 % participants had one visit, 32 % had two visits, 30 % had three visits, and 3 % had four visits. The median duration of follow-up was 3 years (interquartile range (IQR): 0 to 3.6). Table 1 shows the overall cohort characteristics at baseline and by category of 25OHD levels. The median age at enrollment was 10.7 years (IQR: 7.3–14.2), and 62 % were male. African Americans comprised 22 % of the cohort and 14 % reported Hispanic ethnicity. Forty-seven percent of the study population drank milk less often than daily, and only 4 % took nutritional vitamin D supplements. At baseline, the median GFR was 45 (IQR: 34, 58) ml/min per 1.73 m², and the median Up/c ratio was 0.4 (IQR: 0.1, 1) mg/mg. The median iPTH level was 55 (IQR: 31, 101) pg/ml, and the median FGF23 level was 132 (IQR: 88, 209) RU/ml, a value that is 2.3 times higher than the median value of 57 RU/ml in healthy children of comparable age [40].

At enrollment, 28 % of the cohort was 25OHD deficient. Those with 25OHD deficiency were more likely to be of non-white race, have lower maternal education, had assessment of vitamin D level in winter, drank milk less often than daily, spend more time in front of a screen, were older with higher BMI z-scores, had more proteinuria, and higher FGF23 levels, compared to those without 25OHD deficiency.

We found that use of nutritional vitamin D supplements increased from 4 % at enrollment to 13 % 6 years later ($p<0.001$), whereas daily intake of milk decreased from 53 % at baseline to 30 % ($p=0.001$) over this time period. Deficiency of 25OHD was observed in 28 % of the cohort at the baseline visit, 23 % at 2 years and 4 years, and 27 % at 6 years after enrollment.

Predictors of 25OHD deficiency

Table 2 summarizes results of the multivariable logistic regression analysis of predictors of 25OHD deficiency using data from all the visits. African Americans were 15 times more likely to be 25OHD deficient than Caucasians. Older and heavier children were more likely to be deficient. Participants who drank milk less often than daily or did not take nutritional vitamin D supplements were five and nine times more likely to be vitamin D deficient, respectively. Nephrotic range proteinuria was a significant predictor of 25OHD deficiency, whether evaluated as a lagged covariate (OR 8.09, CI 2.38, 27.49, $p=0.001$) or as a concurrent covariate (OR 4.58, CI 1.58–13.26, $p=0.005$). Gender, maternal education status, screen time, GFR, and plasma FGF23 were not significant predictors of 25OHD deficiency.

Predictors of high parathyroid hormone levels

Significant predictors of hyperparathyroidism were low GFR, 25OHD, and serum calcium and high 1,25(OH)₂D and FGF23 levels (Table 3). The odds of hyperparathyroidism were

30 % lower for each 10 ng/ml higher 25OHD level and 45 % lower for each 10 ml/min higher GFR, respectively. Each 1 mg/dl increase in serum calcium was associated with a 51 % lower odds of hyperparathyroidism. The odds of hyperparathyroidism were 4–5 times higher with the highest tertile of FGF23 when compared with the lowest tertile.

Unexpectedly, higher 1,25(OH)₂D levels were associated with a 1.4 times-higher odds of hyperparathyroidism. Use of active vitamin D supplements and serum phosphorus were not significant predictors of hyperparathyroidism.

Determinants of 1,25(OH)₂D levels

1,25(OH)₂D levels were significantly positively associated with serum 25OHD and GFR and inversely associated with age, FGF23 levels, and proteinuria (Table 4). For each 10 ng/ml lower values of 25OHD or 10 ml/min of lower GFR, 1,25(OH)₂D levels were approximately 3 pg/ml and 1 pg/ml lower, respectively. Participants with FGF23 levels in the highest tertile had 1, 25(OH)₂D levels that were approximately 3 pg/ml lower than levels in individuals in the lowest FGF23 tertile. Nephrotic range proteinuria was associated with a 3.6 pg/ml lower 1,25 (OH)₂D level. Sex, race, active vitamin D use, iPTH, serum calcium, and phosphorus were not predictors of 1, 25(OH)₂D levels.

Discussion

Our analysis of markers of mineral metabolism in the largest cohort to date of children with mild to moderate CKD revealed a 28 % prevalence of 25OHD deficiency at enrollment, similar to that reported in prior cross-sectional studies [22, 25]. Deficiency of 25OHD was associated with older age, non-white race, higher BMI, lower milk intake, heavy proteinuria, and non-use of nutritional vitamin D supplements. Some of these findings confirm those previously reported in cross-sectional studies, specifically the association of 25OHD deficiency with non-white race, higher BMI, and heavy proteinuria [22, 25, 27].

We found that non-white race was a significant predictor of 25OHD deficiency, in agreement with multiple prior studies in both children and adults with CKD [21, 22, 25]. Circulating vitamin D is bound to vitamin D binding protein (DBP) (80–90 %) and albumin (10–15 %), with less than 1 % existing in a free, unbound form; free and albumin bound vitamin D constitute the bioavailable 25OHD [41]. In healthy black adults as well as black children with CKD, total 25OHD and DBP levels were lower than in whites; however, bioavailable 25OHD levels were similar between the groups [42, 43]. Thus, it is possible that the greater prevalence of total 25OHD deficiency we found in African Americans may not correspond to lower levels of bioavailable 25OHD when compared to Caucasian children.

Season of blood draw was a significant predictor of vitamin D deficiency, with 25OHD values measured in winter being associated with almost five times-higher risk of deficiency in our study, a finding consistent with previous studies in healthy subjects as well as children with CKD [21, 22, 44, 45]. Although data on sunlight exposure and sunscreen use are currently not available for the CKiD cohort, we evaluated screen time as a surrogate for sunlight exposure. Time spent in front of a screen was not a significant predictor of 25OHD deficiency in the children with CKD in contrast to findings in healthy children [11]. The mean daily screen time was equivalent between children with CKD and healthy children in

NHANES (3.09 ± 1.96 vs. 2.98 ± 1.84 h), suggesting that other factors play a more prominent role in determining deficiency in children with CKD. Of note, the prevalence of 25OHD deficiency in our CKD population was not higher than in the general population. Multiple factors might explain this finding, including the fact that the participants in our study were under medical care and supervision, which may have increased their awareness of the importance of adequate vitamin D levels. Overall use of nutritional vitamin D supplements in the CKiD cohort was low at 8 %, but higher than the 3.3 % supplement use we found in healthy children using NHANES 2001–2004 [11]. Despite the low prevalence of supplement use, we did find an association between lack of supplementation and 25OHD deficiency. In children with CKD, dietary restrictions limit the amount of vitamin D available from diet and limited physical activity leads to decreased sunlight exposure; thus affected children are dependent to a large extent on vitamin D supplementation to maintain adequate levels. Our data corroborated this, as milk intake decreased, but use of nutritional vitamin D supplements increased over time. This latter finding might reflect a response to the revised 2009 KDOQI guidelines, which recommend that 25OHD be measured routinely in CKD patients and supplementation be given if levels are low [46].

Studies in children with CKD have reported variable associations between GFR and 25OHD deficiency [22, 24, 25]. In their study of 182 pediatric CKD patients, Kalkwarf et al. showed that later stages of CKD (4, 5, and 5D) did associate with lower 25OHD concentrations [22]. That study included a significant number of children with moderate to severe CKD and end-stage renal disease; GFR was < 30 ml/min/1.72 m² in 59 % of that cohort. Similarly, Seeherunvong et al. reported that patients with more advanced CKD (stage 3 to 5 with eGFR < 60 ml/min/1.73 m²) had a higher prevalence of vitamin D deficiency than those with more moderately reduced or normal eGFR (42 vs. 26 %; $p < 0.05$) [25]. By contrast, in a study by Stein et al., in which 70 % of children had CKD stage 1 to 3, 25OHD levels did not associate with GFR [24]. We found no association between GFR and 25OHD levels.

We found that lower 25OHD levels were a significant predictor of secondary hyperparathyroidism, findings consistent with studies in children with CKD showing a negative correlation between 25OHD and iPTH levels [22, 23, 25]. These data suggest that 25OHD deficiency contributes to the development of hyperparathyroidism in CKD. Indeed, in children with CKD stages 2 to 4, supplementation with ergocalciferol was associated with delay in the onset of hyperparathyroidism [47]. These findings support the current KDOQI guidelines, which recommend measuring 25OHD levels in CKD stages 2–5 and treating 25OHD deficiency [46]. Further, we and others [48] found that high FGF23 levels were associated with hyperparathyroidism in CKD patients. This association is consistent with studies in experimental CKD showing that FGF23 decreases renal production and serum levels of 1,25(OH)₂D [49], thereby contributing to the development of secondary hyperparathyroidism. Although in experimental non-CKD models FGF23 can directly suppress PTH secretion and gene expression [50], in experimental CKD, FGF23 did not decrease iPTH levels [51]. The apparent resistance to the suppressive effect of FGF23 in CKD has been attributed to down-regulation of the Klotho-FGFR1c receptor complex in the parathyroid glands [51, 52]. Our finding of higher 1, 25(OH)₂D levels being associated with higher odds of hyperparathyroidism is due to the fact that those with hyperparathyroidism are more likely to receive higher doses of calcitriol.

In CKD, 1,25 (OH)₂D levels are dependent on 25OHD availability, and the dependence becomes greater as CKD worsens [22]. We found 25OHD to be a significant predictor of 1,25(OH)₂D levels in multivariable longitudinal analysis, as we had observed earlier using baseline data for the CKiD cohort [40]. FGF23 levels were inversely associated with 1, 25(OH)₂D, consistent with the action of FGF23 to down-regulate renal 1,25 (OH)₂D production [53].

Our study has several limitations. As noted above, we do not have data on sunlight exposure and dietary data is limited to the reported frequency of milk intake. Another significant limitation is the paucity of data on vitamin D doses, as the doses of vitamin D prescribed can vary greatly. However, it is noteworthy that even with variable dosing, any supplement use was associated with less 25(OH)D deficiency than no supplement use. Our study has several strengths, the major one being longitudinal assessment of vitamin D and other markers of mineral metabolism in a large cohort of children with pre-dialysis CKD from multiple centers across North America. In addition, GFR was directly measured in 80 % of this cohort, and the estimating equation used in the remaining subjects is highly accurate [35].

In summary, deficiency of 25OHD is prevalent in children with CKD, is a risk factor for secondary hyperparathyroidism, and is predicted by non-white race, inadequate milk intake, absence of nutritional vitamin D supplementation, higher BMI, and winter season of blood draw. Proteinuria, a CKD-specific risk factor, also was associated with 25OHD deficiency. Intervention trials of vitamin D supplementation, its effects on bioavailable vitamin D levels and other mineral markers, and outcomes such as proteinuria and disease progression are needed in children with CKD.

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Table 1Baseline cohort characteristics by 25-hydroxyvitamin D levels (*N*=506 children)

Characteristic ^a	Overall <i>N</i> =506	25OHD <20 ng/ml <i>N</i> =136	25OHD ≥20 ng/ml <i>N</i> =370	<i>p</i> value ^b
Age at baseline (years)	10.7 (7.3, 14.2)	13.4 (10.0, 15.3)	9.8 (6.4, 13.3)	<0.001
Male gender	62 % (315)	58 % (79)	64 % (236)	0.24
Race				<0.001
Caucasian	67 % (338)	46 % (63)	74 % (275)	
African American	22 % (112)	40 % (55)	15 % (57)	
Other	11 % (56)	13 % (18)	10 % (38)	
Glomerular diagnosis	19 % (97)	39 % (53)	12 % (44)	<0.001
Hispanic ethnicity	14 % (70)	14 % (19)	14 % (51)	0.9
Maternal education>high school	58 % (289)	47 % (64)	63 % (225)	0.002
BMI z-score	0.3 (−0.4, 1.2)	0.7 (−0.2, 1.7)	0.2 (−0.5, 1.0)	<0.001
Blood draw in winter	42 % (213)	57 % (77)	37 % (136)	<0.001
Less often than daily milk consumption	47 % (199)	68 % (79)	39 % (120)	<0.001
Screen time				<0.001
<3 h	51 % (195)	34 % (39)	59 % (156)	
3–4 h	31 % (120)	41 % (48)	27 % (72)	
5 h	18 % (67)	25 % (29)	14 % (38)	
Active vitamin D use	38 % (193)	35 % (47)	39 % (146)	0.31
Nutritional vitamin D use	4 % (18)	2 % (3)	4 % (15)	0.32
GFR (ml/min/1.73 m ²)	44.9 (33.6, 57.9)	46.4 (31.3, 59.9)	44.8 (34.7, 57.5)	0.79
Urinary protein to creatinine ratio (mg/mg of creatinine)	0.4 (0.1, 1.0)	0.7 (0.3, 1.9)	0.3 (0.1, 0.8)	<0.001
Serum calcium (mg/dl)	9.4 (9.1, 9.6)	9.3 (9.1, 9.5)	9.4 (9.2, 9.7)	0.002
Serum phosphorus z-score	0.0 (−0.9, 1.1)	0.2 (−0.9, 1.3)	0.0 (−0.9, 1.0)	0.08
Intact parathyroid hormone (iPTH) (pg/ml)	54.5 (30.9, 100.9)	55.0 (37.0, 146.9)	51.3 (30.7, 92.0)	0.07
Fibroblast growth factor 23 (FGF23) (RU/ml)	132 (88, 209)	154 (95, 246)	125 (85, 194)	0.03

Data are medians [25th, 75th percentiles] or percentage (number)

BMI body mass index, *GFR* glomerular filtration rate

^aMissing data- Hispanic ethnicity: *n*=8; maternal education: *n*=11; BMI z-score: *n*=11; milk consumption: *n*=85; screen time: *n*=124; ieGFR: *n*=83; protein-creatinine ratio: *n*=26; serum calcium and serum phosphorus z-score: *n*=17; intact parathyroid hormone: *n*=202; fibroblast growth factor 23: *n*=54

^bWilcoxon rank sum test for continuous and Chi-square test for categorical variables

Table 2

Multivariable mixed effects logistic regression analysis of determinants of 25OHD deficiency

Covariate	Odds ratio (95 % confidence intervals)	p value
Age, per year	1.23 (1.10, 1.38)	<0.001
Male gender	0.58 (0.29, 1.16)	0.12
Race (reference is Caucasian)		
African American race	15.32 (4.80, 48.86)	<0.001
Others	5.38 (1.67, 17.36)	0.01
Maternal education (>high school vs. high school)	1.60 (0.80, 3.19)	0.19
Body mass index z-score, per 1 standard deviation	1.39 (1.03, 1.87)	0.03
25OHD assessment in winter (vs. summer)	4.78 (2.27, 10.06)	<0.001
Less than daily milk intake (vs. more)	5.31 (2.29, 12.30)	<0.001
Screen time (reference is (<3 h)		
3–4 h	1.90 (0.91, 3.96)	0.09
5 h	1.41 (0.53, 3.76)	0.49
Absence of nutritional vitamin D supplementation	9.46 (1.92, 46.54)	0.01
ieGFR, per 10 ml/min/1.73 m ²	1.00 (0.82, 1.22)	0.9
Proteinuria (mg/mg of creatinine) (reference is p/c ratio<0.5)		
p/c ratio 0.5–1.99	1.82 (0.88, 3.75)	0.11
p/c ratio ≥ 2	8.09 (2.38, 27.49)	0.001
FGF23 tertiles (RU/ml) (reference is first tertile, <102)		
Second tertile (102–174)	1.24 (0.54, 2.86)	0.61
Third tertile (>174)	1.44 (0.59, 3.50)	0.42

eGFR estimated glomerular filtration rate, *FGF23* fibroblast growth factor 23

Table 3

Multivariable mixed-effects logistic regression predictors of hyperparathyroidism

Variable	Odds ratio (95 % confidence intervals)	<i>p</i> value
Age, per year	0.99 (0.93, 1.07)	0.9
Male gender	0.63 (0.35, 1.13)	0.12
Race		
African American	1.49 (0.67, 3.34)	0.32
Other	0.28 (0.11, 0.70)	0.007
Active vitamin D use	1.49 (0.85, 2.64)	0.16
GFR, per 10 ml/min/1.73 m ²	0.55 (0.45, 0.68)	<0.001
Serum 25OHD, per 10 ng/ml	0.72 (0.56, 0.92)	0.009
Serum 1, 25 (OH)2D, per 10 pg/ml	1.39 (1.13, 1.73)	0.002
Phosphorus z-score, per 1 SD	1.09 (0.92, 1.31)	0.29
Serum calcium, per mg/dl	0.49 (0.24, 0.99)	0.05
FGF23 tertiles (RU/ml) (reference is first tertile, < 102)		
Second tertile (102–174)	3.96 (2.09, 7.51)	<0.001
Third tertile (>174)	4.92 (2.40, 10.06)	<0.001

GFR glomerular filtration rate, *FGF23* fibroblast growth factor 23

Table 4Multivariable mixed effects linear regression predictors of 1,25(OH)₂D levels

Variable	Coefficient (95 % confidence interval)	p value
Age, per year	−0.45 (−0.67, −0.22)	<0.001
Male gender	−0.49 (−2.51, 1.52)	0.63
Race		
African American	1.93 (−0.80, 4.67)	0.17
Others	−0.57 (−3.70, 2.57)	0.72
Active vitamin D use	−0.61 (−2.75, 1.52)	0.57
GFR, per 10 ml/min/1.73 m ²	0.91 (0.31, 1.52)	0.003
Serum 25OHD, per 10 ng/ml	2.92 (2.06, 3.77)	<0.001
Intact PTH, per 10 pg/ml	0.03 (−0.08, 0.14)	0.59
FGF23 tertiles (RU/ml) (reference is first tertile, <102)		
Second tertile (102–174)	−1.59 (−3.87, 0.69)	0.17
Third tertile (>174)	−2.93 (−5.57, −0.28)	0.03
Proteinuria (mg/mg of creatinine) (reference is Up/c ratio<0.5)		
Up/c ratio 0.5–1.99	0.47 (−1.63, 2.57)	0.66
Up/c ratio ≥ 2	−3.60 (−7.20, 0.01)	0.05

GFR glomerular filtration rate, *FGF23* fibroblast growth factor 23, *PTH* parathyroid hormone