Vitamin D deficiency and anemia in early chronic kidney disease

Neha M. Patel[1], Orlando M. Gutiérrez[1], Dennis L. Andress[2], Daniel W. Coyne[3], Adeera Levin[4] and Myles Wolf[1]

[1]*Division of Nephrology and Hypertension, Department of Medicine, Miller School of Medicine, University of Miami, Miami, Florida, USA;* [2]*Abbott Laboratories, Research and Development, Abbott Park, Illinois, USA;* [3]*Renal Division, Department of Medicine, Washington University, St Louis, Missouri, USA and* [4]*Division of Nephrology, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada*

Vitamin D has a number of pleiotropic effects in a variety of tissues, in addition to its well-known effects on mineral metabolism. To determine whether it has an effect on erythropoiesis, we studied the association of the components of the vitamin D axis with the prevalence and severity of anemia in chronic kidney disease. We measured the concentrations of 25-hydroxyvitamin D (25D), 1,25-dihydroxyvitamin D (1,25D), and hemoglobin in a cross-sectional study of 1661 subjects in SEEK, a multi-center cohort study of chronic kidney disease patients in the United States, of whom 41% met the criteria for anemia. The mean hemoglobin concentrations significantly decreased with decreasing tertiles of 25D and 1,25D. These linear trends remained significant after adjustment for age, gender, ethnicity, eGFR, diabetes, and parathyroid hormone. In similarly adjusted models, the lowest tertiles of 25D and 1,25D were independently associated with 2.8- and 2.0-fold increased prevalence of anemia compared with their respective highest tertiles. Patients with severe dual deficiency of 25D and 1,25D had a 5.4-fold prevalence of anemia compared with those replete in both. Our study shows that 25D and 1,25D deficiency are independently associated with decreased hemoglobin levels and anemia in chronic kidney disease. Whether this association is causal requires further study.

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Correspondence: *Myles Wolf, Division of Nephrology and Hypertension, Department of Medicine, Miller School of Medicine, University of Miami, 1120 NW 14th Street, Miami, Florida 33136, USA. E-mail: mwolf2@med.miami.edu*

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Chronic kidney disease (CKD) afflicts over 20 million people in the United States.[1] With growing incidence of diabetes mellitus and an aging population, CKD is increasing in incidence and prevalence, and patients continue to suffer from a variety of complications ranging from volume overload and electrolyte imbalances to disordered mineral and bone metabolism and anemia.[1–3] Although principal causes of CKD-associated anemia include erythropoietin deficiency, iron deficiency, and malnutrition–inflammation, recent studies suggest a potential effect of deficiencies in the vitamin D axis as an additional pathophysiological factor.[4–6]

In vitro studies of bone marrow red cell precursor cells demonstrate that calcitriol (1,25-dihydroxyvitamin D (1,25D)) increases erythropoietin-receptor expression and synergistically stimulates proliferation along with erythropoietin.[7] *In vivo* and *in vitro* studies suggest that 1,25D directly affects the proliferation of erythroid precursors via increased membrane permeability of calcium.[8,9] In addition, vitamin D has anti-inflammatory actions that could theoretically improve erythropoietin responsiveness, perhaps by reducing interleukin-6 (IL-6) levels and thus levels of hepcidin, an acute-phase reactant that is a key negative regulator of iron absorption and utilization that is also significantly elevated in CKD.[10,11] Finally, extremely high parathyroid hormone (PTH) levels have been considered a mechanism for decreased erythropoiesis via increased bone marrow fibrosis and erythropoietin resistance in CKD patients.[12,13] Whether this represents exclusively a direct antagonistic effect of PTH on erythropoiesis or could be partially owing to the low 1,25D levels that accompany high PTH in dialysis patients, is unknown because vitamin D levels were not measured in the studies that examined PTH and anemia.[4,14] Indeed, previous studies have suggested improved control of anemia in dialysis patients treated with active forms of vitamin D such as calcitriol or nutritional vitamin D precursors such as ergocalciferol.[13,15–17]

Given these associations, the high prevalence of anemia and 25-hydroxyvitamin D (25D) and 1,25D deficiency in CKD, and the abundant availability of therapies for correcting these deficiencies, we undertook a cross-sectional

analysis of a cohort of pre-dialysis CKD patients to test the hypothesis that deficiencies in the vitamin D axis, both 25D and 1,25D, are independently associated with lower hemoglobin levels and higher prevalence of anemia in early CKD.

RESULTS

Subject characteristics

In the overall study sample, the mean age was 70 ± 11 years, mean estimated glomerular filtration rate (eGFR) was 47 ± 18 ml/min/1.73 m^2, and mean hemoglobin was 13 ± 2 g/dl. The criteria for anemia (hemoglobin <13.5 g/dl for men and <12 for women) were met by 41% of subjects. The clinical and demographic characteristics of anemic versus non-anemic subjects are compared in Table 1. Compared with non-anemic subjects, anemic subjects were more likely to be Black, older males, with a history of diabetes, congestive heart failure, and hypertension, and were more likely to have been treated with angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin-II receptor blockers (ARBs). In addition, anemic subjects had lower GFR, serum albumin, calcium, 25D, and 1,25D, and higher serum phosphorus and PTH than non-anemic subjects. While median IL-6 levels were significantly higher in the anemic patients as compared with that in the non-anemic group (3.6 pg/ml, interquartile range: 2.6–5.7 versus 3.0 pg/ml, interquartile range: 2.2–4.5; $P=0.004$), there was no significant difference between the median C-reactive protein (CRP) levels in anemic versus non-anemic patients (2.7 mg/l, interquartile range: 1.2–7.9 versus 2.8 mg/l, interquartile range: 1.2–6.4; $P=0.67$).

Table 1 | Characteristics of the study population by anemia status

Characteristic	Anemic	Non-anemic	*P*-value
n	680	981	
Age (years)	71 ± 11	69 ± 11	0.003
Female gender (%)	41	60	<0.001
Black race (%)	17	8	<0.001
Body mass index (kg/m^2)	31 ± 7	31 ± 7	0.53
Blood pressure (mm Hg)			
Systolic	132 ± 18	131 ± 17	0.26
Diastolic	71 ± 9	74 ± 11	<0.001
Estimated GFR (ml/min/1.73 m^2)	41 ± 16	52 ± 17	<0.001
Chronic kidney disease stage (%)			
2 (eGFR >60)	12	31	<0.001
3 (eGFR 30–59)	62	59	<0.001
4 (eGFR 15–29)	23	9	<0.001
5 (eGFR <15)	3	1	<0.001
Laboratory results			
Hemoglobin, men (g/dl)	12 ± 1	15 ± 1	<0.001
Hemoglobin, women (g/dl)	11 ± 0.8	13 ± 1	<0.001
25D (ng/ml)	26 ± 13	31 ± 12	<0.001
1,25D (pg/ml)	24 (15, 33)	32 (22, 45)	<0.001
Albumin (g/dl)	4.1 ± 0.4	4.3 ± 0.3	< 0.001
Calcium (mg/dl)[a]	9.1 ± 0.5	9.2 ± 0.4	<0.001
Phosphorus (mg/dl)	3.8 ± 0.7	3.6 ± 0.6	<0.001
PTH (pg/ml)	68 (42, 114)	50 (33, 78)	<0.001
Co-morbidities (%)			
Diabetes mellitus	58	42	<0.001
Hypertension	92	83	<0.001
Congestive heart failure	26	15	<0.001
Medications			
ACE inhibitor	44	34	<0.001
ARB	37	32	0.03

Abbreviations: 25D, 25-hydroxyvitamin D; 1,25D, calcitriol/1,25-dihydroxyvitamin D; ACE, angiotensin-converting enzyme; ARB, angiotensin-II-receptor blocker; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.
Anemia was defined as a hemoglobin value of <13.5 g/dl for men and <12 g/dl for women.
[a]Corrected for serum albumin.

Relationship between vitamin D levels and hemoglobin concentration

There was a linear relationship between hemoglobin concentration and both 25D ($r=0.22$; $P<0.001$) and 1,25D ($r=0.26$; $P<0.001$) concentrations. Mean hemoglobin concentrations decreased with decreasing tertiles of 25D and 1,25D (Table 2). In multivariable linear regression model adjusted for age, sex, race, eGFR, diabetes, and PTH, there was a stepwise decrease in hemoglobin concentrations with decreasing tertiles of 25D (Table 2). Interestingly, the magnitude of effect was greatest among subjects who had dual deficiency of both vitamin D metabolites—subjects with severe deficiency of both forms of vitamin D (25D <10 ng/ml and 1,25D <30 pg/ml) had a multivariable-adjusted 8% lower hemoglobin concentration when compared with subjects who had the highest concentrations of both 25D (>30 ng/ml) and 1,25D (45 pg/ml). Furthermore, multivariate analyses adjusted for all other covariates in addition to both vitamin D metabolites simultaneously showed independent effects of 25D and 1,25D on hemoglobin concentration ($P<0.001$ for both).

Table 2 | Mean hemoglobin concentrations by tertiles of 25D and 1,25D, and multivariable-adjusted[a] Δ-hemoglobin comparing lower tertiles to the highest

	25-Hydroxyvitamin D			
	Tertile 1 (<10 ng/ml)	Tertile 2 (10–30 ng/ml)	Tertile 3 (>30 ng/ml)	*P*
n	52	911	708	
Mean hemoglobin (g/dl)	11.7 ± 2	12.8 ± 2	13.5 ± 2	<0.001
Δ Hemoglobin (%)	−6%	−2%	Reference	<0.01
	1,25-Dihydroxyvitamin D			
	Tertile 1 (<30 pg/ml)	Tertile 2 (30–45 pg/ml)	Tertile 3 (>45 pg/ml)	*P*
n	897	456	308	
Mean hemoglobin (g/dl)	12.7 ± 2	13.3 ± 2	13.7 ± 2	<0.001
Δ Hemoglobin (%)	−3%	−2%	Reference	<0.01

Abbreviations: 25D, 25-hydroxyvitamin D; 1,25D, calcitriol/1,25-dihydroxyvitamin D; BMI, body mass index; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.
[a]Adjusted for age, race, gender, eGFR, diabetes, hypertension, BMI, PTH.

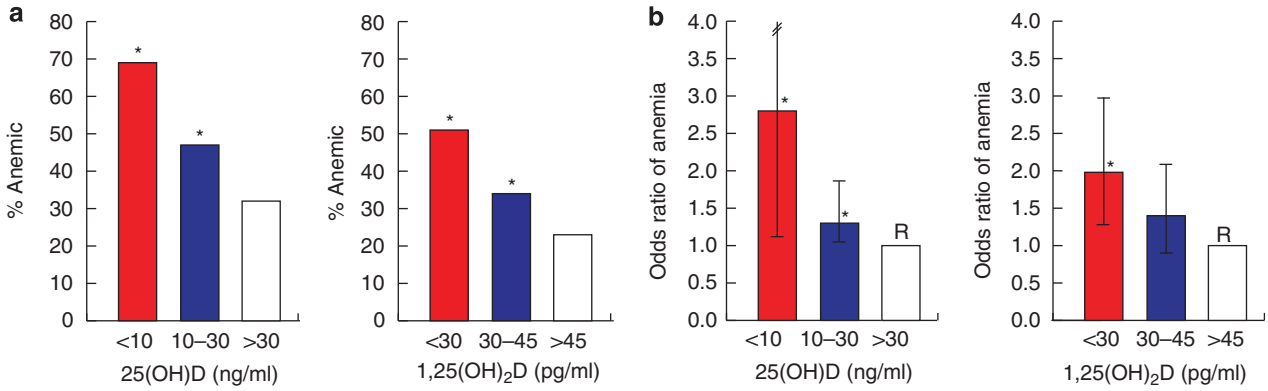

Figure 1 | Prevalence and odds ratios of anemia according to tertiles of 25D and 1,25D. (**a**) Prevalence of anemia by tertiles of 25D and 1,25D. (**b**) Multivariable-adjusted odds ratios of anemia by tertiles of 25D and 1,25D. *$P<0.05$ compared with the highest tertile. Adjusted for age, race, gender, eGFR, diabetes, hypertension, body mass index, and PTH.

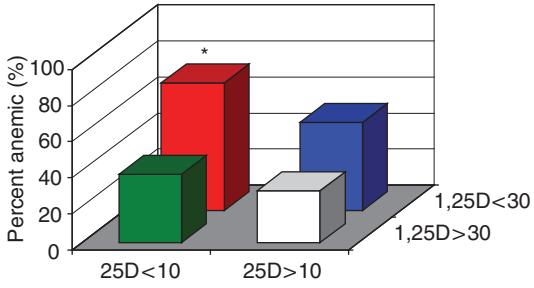

Figure 2 | The combined effects of lowest 25D and 1,25D levels on the prevalence of anemia. *$P<0.01$ compared with the high 25D and high 1,25D group.

Relationship between vitamin D levels and prevalence of anemia

The lowest tertiles of 25D and 1,25D were associated with the highest prevalence of anemia, and the middle tertiles of each were associated with an intermediate prevalence (Figure 1a). In multivariable-adjusted logistic regression models (Figure 1b), the lowest tertile of 25D was associated with 2.8-fold increased prevalence of anemia (95% confidence interval 1.5–5.1) compared with the highest, while the lowest tertile of 1,25D was associated with 2.0-fold increased prevalence of anemia (95% confidence interval 1.5–2.9) compared with the highest tertile. Additionally, subjects with severe deficiency of both forms of vitamin D (25D <10 ng/ml and 1,25D <30 pg/ml) had the greatest prevalence of anemia compared with subjects with a deficiency in a single vitamin D metabolite or those with sufficient levels of both (Figure 2). In the corresponding multivariable analysis, subjects with severe dual deficiency of both vitamin D metabolites had a 5.4-fold increased prevalence of anemia (95% confidence interval 1.9, 15.1) compared with subjects who had both 25D levels above 30 ng/ml and 1,25D levels above 45 pg/ml.

Effect of renal function, gender, medication use, and markers of inflammation

The independent associations between vitamin D concentrations (both 25D and 1,25D) and hemoglobin concentrations were consistent across all stages of CKD—when adjusting for residual confounding of eGFR, all subsets of the CKD population under study demonstrated a graded, stepwise decrease in hemoglobin levels and increased prevalence of anemia with decreasing vitamin D levels. The results were not modified by gender or by use of ACE inhibitors or ARBs (data not shown).

In a secondary analysis of the 370 subjects with available measurements of CRP and IL-6, the effects of 25D and 1,25D on hemoglobin concentration and prevalence of anemia remained significant after adjusting for the inflammatory biomarkers.

DISCUSSION

In this cross-sectional study, we observed a linear relationship between vitamin D and hemoglobin concentrations. There was a stepwise decrease in hemoglobin concentrations and a stepwise increase in the prevalence of anemia with decreasing tertiles of vitamin D concentrations that persisted in multivariable analyses. Furthermore, there was an additive effect of dual deficiency of 25D and 1,25D on the prevalence of anemia. Each of these associations was independent of multiple potential confounding factors and was not modified by gender, use of ACE inhibitors, or ARBs, or inflammatory markers such as CRP and IL-6. Collectively, these data suggest that deficiencies in the vitamin D axis are potential novel risk factors for anemia in CKD. Although causality can only be determined through confirmatory longitudinal observational and subsequent interventional studies, the results suggest that an additional pleiotropic benefit of vitamin D therapy in CKD might be to attenuate anemia and improve sensitivity to erythropoietin.

The results of this study are consistent with previous studies of dialysis patients in which vitamin D supplementation with ergocalciferol and calcitriol appeared to increase sensitivity to erythropoietin as evidenced by a lower requirement for erythropoiesis-stimulating agents to achieve similar control of anemia.[13,15–17] Additionally, in a recent cross-sectional analysis of the Third National Health and

Nutrition Examination Study (NHANES III), lower 25D levels were independently associated with lower hemoglobin concentrations within the CKD population.[6] We confirm these results in this study, but also extend them in two important ways. First, unlike the NHANES study, we were able to further adjust for PTH levels and data points regarding treatment with ACE inhibitors, and ARBs, that were not available in NHANES III. While it is encouraging that the results remained significant, by observing an association that is independent of PTH, we can speculate that at least a portion of the erythropoietin resistance historically attributed to marked increases in PTH may in fact relate to deficiencies in the vitamin D axis that were not assessed in previous studies. Second, unlike previous reports, we were able to assess 1,25D concentrations and demonstrate their independent association with anemia as well. Furthermore, the seemingly additive effect of a combination of 25D and 1,25D deficiency, that is, global deficiency in the vitamin D axis, lends overall support for the role of the vitamin D axis in contributing to normal erythropoiesis in this population.

Cardiovascular disease is highly prevalent in the CKD population when compared with the general population and remains the leading cause of death.[18] In addition to traditional risk factors, deficiencies in the vitamin D axis and anemia have also been implicated in the excessive cardiovascular risk associated with CKD.[19] Interestingly, one of the main cardiovascular disease phenotypes that is strongly linked to mortality and that is associated with both deficiencies in the vitamin D axis and anemia, is left ventricular hypertrophy.[20,21] Thus, it is interesting to speculate whether there could be mechanistic overlap between the two, and whether vitamin D therapy used as an adjunct to traditional anemia management might together improve outcomes by attenuating cardiovascular risk. Although a number of clinical trials have failed to demonstrate a reduction in cardiovascular risk in response to anemia management in CKD,[22,23] observational studies have demonstrated clear links between higher hemoglobin levels and improved survival in the CKD population.[24–26]

There are several important limitations to this study. Due to the study's cross-sectional design, it is not possible to determine a causal relationship between vitamin D deficiency and anemia. In addition, data on iron stores and treatment with erythropoiesis stimulating agents (ESAs) were unavailable. However, previous studies that did correct for ESA use[13,15] and iron stores[6] found similar results. Furthermore, it is unlikely that more than a small proportion of this population of CKD patients, most of which had early stages of CKD and was largely recruited from primary care and family practices, would have been on ESAs during the study. We did have data on CRP and IL-6 for a subset of patients, and the relationship between vitamin D and hemoglobin concentrations remained significant after adjusting for these indices. Due to the small sample size of this exploratory analysis, additional studies with larger numbers of participants are needed to further examine the potential relationships between vitamin D, inflammation, iron utilization, and erythropoiesis.

Given the high prevalence of anemia and deficiencies in the vitamin D axis in patients with CKD, and the ease and low cost of correcting these deficiencies, this study in the context of other findings and biological plausibility suggests a potential additional benefit to early vitamin D treatment as an adjunct to traditional anemia management. Since both 25D and 1,25D deficiency are independently associated with lower hemoglobin concentration, future studies should explore the relative erythropoiesis-stimulating effects of nutritional vitamin D supplements and active forms of vitamin D, alone and in combination.

MATERIALS AND METHODS

Study population

The Study to Evaluate Early Kidney Disease (SEEK) was a multicenter, descriptive study designed to assess the timing and severity of disordered mineral metabolism across the spectrum of CKD.[27] Patients greater than 40 years of age with an established healthcare provider and a screening eGFR <60 ml/min/1.73 m^2 as determined by the Modification of Diet in Renal Disease equation,[28] were eligible for enrollment. The eGFR was recalculated at the subsequent study visit; hence some subjects had eGFR >60 ml/min/1.73 m^2 on repeat testing but remained in the study. The exclusion criteria included renal replacement therapy, a history of primary parathyroid disease, or use of prescription-based vitamin D supplementation within 12 months of screening. A total of 1,814 eligible patients were recruited from 153 centers across all regions of the United States. Study visits were conducted during peak sunlight season between June 2004 and October 2004, thereby minimizing confounding due to seasonal variations in sun exposure. Of the 1,814 patients included in the SEEK study, 153 were excluded because of missing data on 25D, 1,25D, or hemoglobin concentrations, leaving 1,661 patients in the final study sample.

Variables of interest

The key variables of interest for this study were hemoglobin, 25D, and 1,25D. The primary outcome was presence of anemia, defined as hemoglobin <13.5 g/dl for men and <12 g/dl for women.[29] The primary predictors were 25D and 1,25D levels, which were measured using DiaSorin radioimmunoassay kits (Stillwater, MN, USA). 25D deficiency was defined as a level of <30 ng/ml, while severe 25D deficiency was defined as a level of <10 ng/ml as has been done previously.[19] Since normal ranges for 1,25D are not clearly established, we analyzed the tertiles of 1,25D levels according to their distribution in subjects with sufficient renal function (eGFR >60 ml/min/1.73 m^2). We defined the lowest tertile as 1,25D deficient (<30 pg/ml) and the uppermost tertile of 1,25D as >45 pg/ml.

Covariates included age, gender, race, body mass index, blood pressure, laboratory tests (eGFR, calcium, phosphorus, parathyroid hormone), co-morbidities (diabetes mellitus, hypertension, congestive heart failure), and medication use (ACE inhibitors, ARBs).

All samples were analyzed at a central laboratory (Quintiles Laboratories Ltd, Parsippany, NJ, USA) by technicians who were blinded to clinical and demographic data. Calcium, phosphorus, and creatinine levels were quantified with a standard autoanalyzer. Total calcium was corrected for serum albumin using the following formula: $\text{Calcium} = \text{Ca} + 0.8 \times (4.0 - \text{Albumin})$ when albumin was

less than 4.0 g/dl. Intact PTH was quantified with a chemiluminescence assay (DPC, Los Angeles, CA, USA).

Statistical analyses

The clinical and demographic data were compared between anemic and non-anemic subjects using Student's *t*-test, Wilcoxon's rank-sum test, or χ^2-test as appropriate. Linear regression models were used to examine the relationships between vitamin D and hemoglobin concentration. We used multivariable models to adjust for age, gender, race, eGFR, diabetes, hypertension, body mass index, and PTH. Univariable- and multivariable-adjusted logistic regression models were used to examine the association between vitamin D levels and prevalence of anemia. In all models, vitamin D concentrations were examined on a continuous scale, and also categorically in tertiles. To investigate whether there were additive effects of 25D and 1,25D deficiency on hemoglobin concentrations and prevalence of anemia, we repeated the multivariable analyses after creating four groups of subjects based on the permutations of presence or absence of severe 25D (<10 ng/ml) and 1,25D (<30 pg/ml) deficiency. To investigate the independent effects of 25D and 1,25D deficiency on hemoglobin concentration, we performed additional multivariable-adjusted analyses including all other covariates plus both vitamin D metabolites simultaneously.

To investigate whether the effects of vitamin D levels on hemoglobin were independent of renal function, we performed additional stratified analyses within individual stages of CKD. We also adjusted for use of ACE inhibitors and ARBs in further analyses given prior reports of increased human erythropoietin requirements in hemodialysis patients prescribed ACE inhibitors and/or ARBs.[30,31] Finally, we performed a secondary exploratory analysis of the effect brought on the association between vitamin D levels and anemia by the markers of inflammation, CRP and IL-6, which were available in a subset of 370 subjects. All statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC, USA), and a *P*-value <0.05 was considered statistically significant.

DISCLOSURE

AL is a consultant for Abbott, Amgen, Genzyme, Shire, Ortho, and Roche. DWC has received research support from Abbott and Amgen, and honoraria from Abbott, Amgen, and INEOS. DLA is an employee of Abbott. OMG has received consulting fees and honoraria from Abbott. MW has received research support from Shire and honoraria from Abbott, Amgen, Genzyme, and Shire.

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