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Vitamin D levels and early mortality among incident hemodialysis patients

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Vitamin D deficiency is associated with cardiovascular disease, the most common cause of mortality in hemodialysis patients. To investigate the relation between blood levels of 25-hydroxyvitamin D (25D) and 1,25-dihydroxyvitamin D (1,25D) with hemodialysis outcomes, we measured baseline vitamin D levels in a cross-sectional analysis of 825 consecutive patients from within a prospective cohort of incident US hemodialysis patients. Of these patients, 78% were considered vitamin D deficient with 18% considered severely deficient. Calcium, phosphorus, and parathyroid hormone levels correlated poorly with 25D and 1,25D concentrations. To test the association between baseline vitamin D levels and 90-day mortality, we selected the next 175 consecutive participants who died within 90 days and compared them to the 750 patients who survived in a nested case–control analysis. While low vitamin D levels were associated with increased mortality, significant interaction was noted between vitamin D levels, subsequent active vitamin D therapy, and survival. Compared to patients with the highest 25D or 1,25D levels who received therapy, untreated deficient patients were at significantly increased risk for early mortality. Our study shows that among incident hemodialysis patients, vitamin D deficiency is common, correlates poorly with other components of mineral metabolism and is associated with increased early mortality.

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KEYWORDS: vitamin D; calcitriol; kidney disease

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Vitamin D deficiency is common in hospitalized and otherwise healthy individuals throughout the world.[1–8] Maintaining adequate blood levels of 25-hydroxyvitamin D (25D), the storage form of vitamin D, requires sufficient *de novo* cutaneous synthesis stimulated by ultraviolet radiation or adequate dietary intake of fortified foods and nutritional supplements.[9,10] The kidney converts 25D to its biologically active hormonal form, 1,25-dihydroxyvitamin D (1,25D), which binds nuclear receptors that regulate gene transcription.[11,12] Since the 17th century, clinicians have been familiar with the consequences of severe vitamin D deficiency in the form of rickets with its accompanying musculoskeletal and biochemical abnormalities and its associated increased risk for early death.[2,13,14] In the current era, even with the potential resurgence of rickets,[14] severe vitamin D deficiency is less common. Nevertheless, given the ubiquitous tissue distribution of the vitamin D receptor, mild to moderate vitamin D deficiency has been linked to diabetes, malignancy, neurological disorders, hypertension, and congestive heart failure in selected populations, although the results have been inconsistent.[15–22]

Patients with kidney disease are at high risk for 25D deficiency and, unlike non-kidney disease patients who are able to tightly regulate 1,25D levels within the normal range, they also demonstrate profound reductions in 1,25D levels.[23–27] Following the initiation of dialysis, patients with kidney disease are at dramatically increased risk of early mortality.[28] The combination of significant 25D deficiency, impaired conversion of 25D to 1,25D, and high early mortality rates render dialysis patients the ideal model to study the impact of vitamin D deficiency on mortality in the current era. Few, if any, studies have examined the potential link between vitamin D deficiency and mortality on hemodialysis. We and others have reported that therapy with activated vitamin D is independently associated with improved survival among patients initiating hemodialysis.[29–34] Although these observational studies are subject to bias and confounding, they suggest that decreased endogenous vitamin D levels may be linked to increased mortality. We therefore examined the prevalence of vitamin D deficiency in

a cross-section of US patients at the time they initiated chronic hemodialysis and, in a case–control sample of patients nested within a prospective cohort, tested the hypothesis that decreased levels of 25D and 1,25D before treatment with any active vitamin D are associated with increased risk for early mortality.

RESULTS

Demographics and circulating 25D and 1,25D levels

The initial cross-sectional study sample included 825 consecutive patients from 569 unique hemodialysis centers in 37 states. Their baseline characteristics at the initiation of dialysis are presented in Table 1. Distributions of 25D and 1,25D levels are shown in Figure 1. The mean level of 25D was 21 ± 13 ng/ml. Only 22% of subjects had 25D levels >30 ng/ml, whereas 60% had levels between 10 and 30 ng/ml; the remaining 18% were severely vitamin D deficient (<10 ng/ml). Compared with men, women were more likely to be severely 25D deficient (23 *vs* 15%; $P<0.01$). Compared with white subjects, black subjects had significantly lower mean 25D levels (17 ± 10 *vs* 24 ± 14 ng/ml; $P<0.01$) and were more likely to be severely 25D deficient (31 *vs* 12%; $P<0.01$). Compared to patients without diabetes, those with diabetes were more likely to be severely 25D deficient (22 *vs* 17%; $P<0.01$). The mean level of 1,25D was 11 ± 10 pg/ml. Serum 1,25D levels did not differ between men and women, white and black subjects, or between patients with and without diabetes. As expected, both 25D and 1,25D levels were significantly greater among patients who initiated dialysis in the summer compared with those who initiated in the winter (25D: 25 ± 14 *vs* 19 ± 11 ng/ml; $P<0.01$; 1,25D: 13 ± 11 *vs* 10 ± 9 pg/ml; $P<0.01$).

25D levels correlated weakly with serum levels of calcium ($r=0.18$), parathyroid hormone (PTH) ($r=-0.14$), and albumin ($r=0.31$); there was no correlation with serum phosphorus or creatinine. Table 2a illustrates mineral and nutrition metabolites according to the 25D groups. Subjects with vitamin D deficiency had slightly lower serum calcium and albumin levels, and increased PTH and alkaline phosphatase levels compared to subjects with normal 25D

Table 1 | Baseline characteristics of the initial 825 consecutive study participants

Demographic characteristics	
Age (years)	63 ± 15
Gender (% women)	47
Race (%)	
White	60
Black	32
Other	8
Etiology of renal failure (%)	
Diabetes mellitus	43
Hypertension	34
Glomerulonephritis	10
Polycystic kidney disease	3
Other	10
Mean arterial pressure (mm Hg)	98 ± 16
Body mass index (kg/m^2)	27.3 ± 6.8
Access at the initiation of hemodialysis (%)	
Arteriovenous fistula	27
Arteriovenous graft	12
Catheter	61
Comorbid conditions (%)	
Coronary artery disease/myocardial infarction	16
Congestive heart failure	21
Peripheral vascular disease	10
Stroke	4
Hyperlipidemia	18
Malignancy	4
Chronic obstructive pulmonary disease	4
Baseline laboratory test results	
Calcium (mg/dl)	8.5 ± 0.8
Phosphorus (mg/dl)	4.6 ± 1.6
Parathyroid hormone (bio-intact; pg/ml)	193 (105, 341)
Albumin (g/dl)	3.5 ± 0.5
Creatinine (mg/dl)	6.3 ± 2.7
Hemoglobin (g/l)	10.3 ± 1.4

Results are expressed as mean $\pm$ s.d. or medians (interquartile ranges) as appropriate.

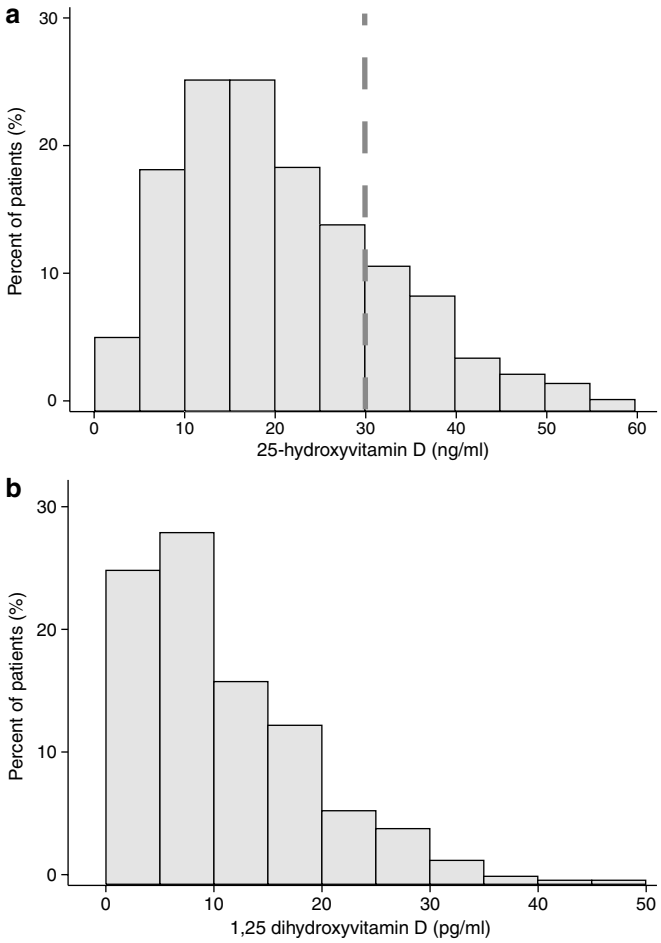

Figure 1 | Distribution of vitamin D levels among 825 incident hemodialysis patients. (**a**) 25-hydroxyvitamin D and (**b**) 1,25-dihydroxyvitamin D (25D). The vertical dashed line (**a**) indicates the lower limit of the normal range for 25D of 30 ng/ml.

Table 2a | Markers of mineral metabolism, nutrition, and renal function according to serum 25D levels

	Severely deficient <10 ng/ml *n*=147	Deficient 10–30 ng/ml *n*=472	Replete >30 ng/ml *n*=173	*P*-values
Calcium (mg/dl)	8.3±0.8	8.4±0.8	8.7±0.8	<0.01
Phosphorus (mg/dl)	4.7±1.6	4.6±1.5	4.7±1.6	NS
PTH (pg/ml)	250 (139, 386)	195 (105, 343)	159 (81, 258)	<0.01
Alkaline phosphatase (U/l)	92 (70, 124)	83 (65, 112)	84 (66, 107)	0.03
Albumin (g/dl)	3.2±0.6	3.5±0.5	3.7±0.5	<0.01
Creatinine (mg/dl)	6.2±2.4	6.3±2.7	6.2±2.9	NS

25D, 25-hydroxyvitamin D; NS, nonsignificant; PTH, parathyroid hormone.
Results are expressed as mean±s.d. or medians (interquartile ranges) as appropriate.

Table 2b | Markers of mineral metabolism, nutrition, and renal function according to serum 1,25D levels

	Tertile 1 <6 pg/ml *n*=184	Tertile 2 6–13 pg/ml *n*=234	Tertile 3 >13 pg/ml *n*=188	*P*-values
Calcium (mg/dl)	8.3±0.9	8.5±0.8	8.6±0.8	<0.01
Phosphorus (mg/dl)	4.8±1.7	4.6±1.6	4.6±1.5	NS
PTH (pg/ml)	183 (103, 300)	207 (112, 377)	181 (91, 301)	NS
Alkaline phosphatase (U/l)	86 (62, 114)	84 (67, 116)	83 (67, 109)	NS
Albumin (g/dl)	3.3±0.6	3.5±0.5	3.6±0.4	<0.01
Creatinine (mg/dl)	6.5±2.8	6.0±2.5	6.1±3.0	NS

1,25D, 1,25-dihydroxyvitamin D; NS, nonsignificant; PTH, parathyroid hormone.
Results are expressed as mean±s.d. or medians (interquartile ranges) as appropriate.

stores. Nevertheless, 79% of patients with serum PTH <150 pg/ml were vitamin D deficient with 25D levels <30 ng/ml.

The strongest correlate of 1,25D levels was the serum 25D ($r=0.31$). Other factors that correlated with 1,25D included serum calcium ($r=0.12$) and albumin ($r=0.21$); there was no correlation with PTH, phosphorus, or creatinine levels. Table 2b illustrates mineral and nutrition metabolites according to tertiles of serum 1,25D. Compared to subjects in the upper 1,25D tertile, those in the lower tertiles had decreased serum calcium and albumin levels. There was no association between 1,25D tertiles and serum phosphorus, PTH, alkaline phosphatase, or creatinine.

The median PTH was 193 pg/ml; 17% had PTH levels <75 pg/ml, 20% were 75–150 pg/ml, and 63% were >150 pg/ml. Compared to subjects with PTH>150 pg/ml, those with levels ⩽150 pg/ml had no significant difference in median 1,25D levels (12±11 *vs* 11±10; $P=0.9$) but significantly increased mean 25D levels (24±12 *vs* 20±13; $P<0.01$). Nonetheless, there was substantial overlap in the distributions of 25D and 1,25D levels according to baseline PTH levels as shown in Figure 2a and b.

Circulating 25D, 1,25D, and survival on hemodialysis

The case–control sample of 1000 patients was used to study survival. Baseline characteristics according to 90-day outcomes are presented in Table 3. Factors that were associated with early mortality in this study were similar to those reported in larger populations initiating hemodialysis in the United States.[35] In the crude analyses, there were no significant differences in mean levels of 25D (21±12 *vs* 21±13 ng/ml; $P=0.50$) or 1,25D (10±8 *vs* 11±10 pg/ml; $P=0.12$) comparing subjects who died with those who survived. However, subjects with severe 25D deficiency (<10 ng/ml) were at significantly increased risk of all-cause mortality compared to subjects with normal 25D levels

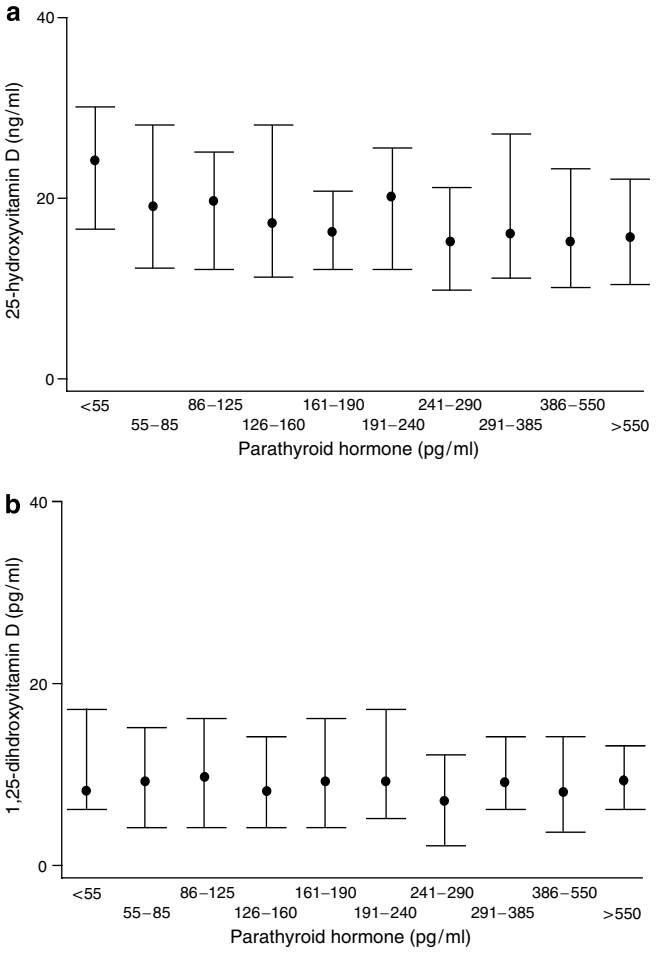

Figure 2 | Distributions of vitamin D levels according to baseline PTH levels. (**a**) 25-hydroxyvitamin D ($n=792$) and (**b**) 1,25-dihydroxyvitamin D ($n=606$). PTH levels are divided into groups according to the ascending 10th percentiles of the distribution in the entire study population. Mean vitamin D levels are represented by the central point and the bars represent interquartile ranges (25th and 75th percentiles).

Table 3 | Baseline characteristics of patients who survived or died within the first 90 days of initiating hemodialysis

	Survived *n*=750	Died *n*=250	*P*-values
Demographic characteristics			
Age (years)	62±16	71±13	<0.01
Gender (% women)	47	46	NS
Race (%)			<0.01
White	59	72	
Black	33	24	
Other	8	4	
Etiology of renal failure (%)			NS
Diabetes mellitus	43	43	
Hypertension	34	37	
Glomerulonephritis	10	6	
Polycystic kidney disease	3	1	
Other	10	13	
Mean arterial pressure (mm Hg)	99±15	89±22	<0.01
Body mass index (kg/m^2)	27.3±6.7	27.0±7.5	NS
Access at the initiation of hemodialysis (%)			<0.01
Arteriovenous fistula	28	9	
Arteriovenous graft	13	7	
Catheter	59	84	
Comorbid conditions (%)			
Coronary artery disease/myocardial infarction	16	18	NS
Congestive heart failure	19	30	<0.01
Peripheral vascular disease	10	12	NS
Stroke	4	7	0.01
Hyperlipidemia	18	12	0.01
Malignancy	4	8	0.01
Chronic obstructive pulmonary disease	4	5	NS
Baseline laboratory test results			
Calcium (mg/dl)	8.5±0.8	8.4±0.8	NS
Phosphorus (mg/dl)	4.7±1.6	4.3±0.9	<0.01
Parathyroid hormone (intact; pg/ml)	199 (111, 341)	166 (92, 297)	<0.01
Albumin (g/dl)	3.5±0.5	3.2±0.6	<0.01
Creatinine (mg/dl)	6.3±2.7	5.3±2.3	<0.01
Hemoglobin (g/l)	10.3±1.4	10.4±1.3	NS

NS, nonsignificant.
Results are expressed as mean±s.d. or medians (interquartile ranges) as appropriate.

Table 4a | ORs of risk of death within 90 days of initiating chronic hemodialysis according to baseline 25D levels

25D	<10 ng/ml *n*=187	10–30 ng/ml *n*=594	>30 ng/ml *n*=203
All-cause mortality			
Age, gender, race-adjusted	1.9* (1.3–2.9)	1.4* (1.0–2.0)	1.0 (REF)
Multivariate-adjusted	1.6* (1.0–2.4)	1.3 (0.9–1.8)	1.0 (REF)
Cardiovascular mortality			
Age, gender, race-adjusted	1.9* (1.0–3.4)	1.8* (1.2–2.9)	1.0 (REF)
Multivariate-adjusted	1.6 (0.8–3.0)	1.6* (1.0–2.6)	1.0 (REF)

25D, 25-hydroxyvitamin D; OR, odds ratio.
*$P<0.05$ compared with the reference group.
REF refers to the reference group for these analyses.

(Table 4a). There was a statistically significant increased risk of early mortality in subjects with 1,25D levels of 6–13 compared with >13 pg/ml but no significant increased risk in the <6 pg/ml group (Table 4b).

There was no significant interaction between PTH, vitamin D levels, and survival, modeling PTH either on a continuous scale or categorically as ⩽or >150 pg/ml. Compared to no treatment, subsequent therapy with activated vitamin D was associated with a multivariate-adjusted survival advantage (odds ratio (OR) 0.60; 95% confidence interval (CI) 0.37, 0.91; $P<0.01$). Given the effect of active vitamin D therapy on outcome, we formally tested for and identified effect modification between active vitamin D therapy, endogenous vitamin D levels, and survival

Table 4b | ORs of risk of death within 90 days of initiating chronic hemodialysis according to baseline 1,25D levels

1,25D	<6 pg/ml *n*=220	6–13 pg/ml *n*=253	>13 pg/ml *n*=246
All-cause mortality			
Age, gender, race-adjusted	1.5 (0.9–2.4)	1.7* (1.1–2.7)	1.0 (REF)
Multivariate-adjusted	1.4 (0.8–2.3)	1.8* (1.1–2.9)	1.0 (REF)
Cardiovascular mortality			
Age, gender, race-adjusted	1.6 (0.9–2.8)	1.6 (0.9–2.8)	1.0 (REF)
Multivariate-adjusted	1.5 (0.8–2.9)	1.8 (0.9–3.4)	1.0 (REF)

1,25D, 1,25-dihydroxyvitamin D; OR, odds ratio.
*$P<0.05$ compared with the reference group.
REF refers to the reference group for these analyses.

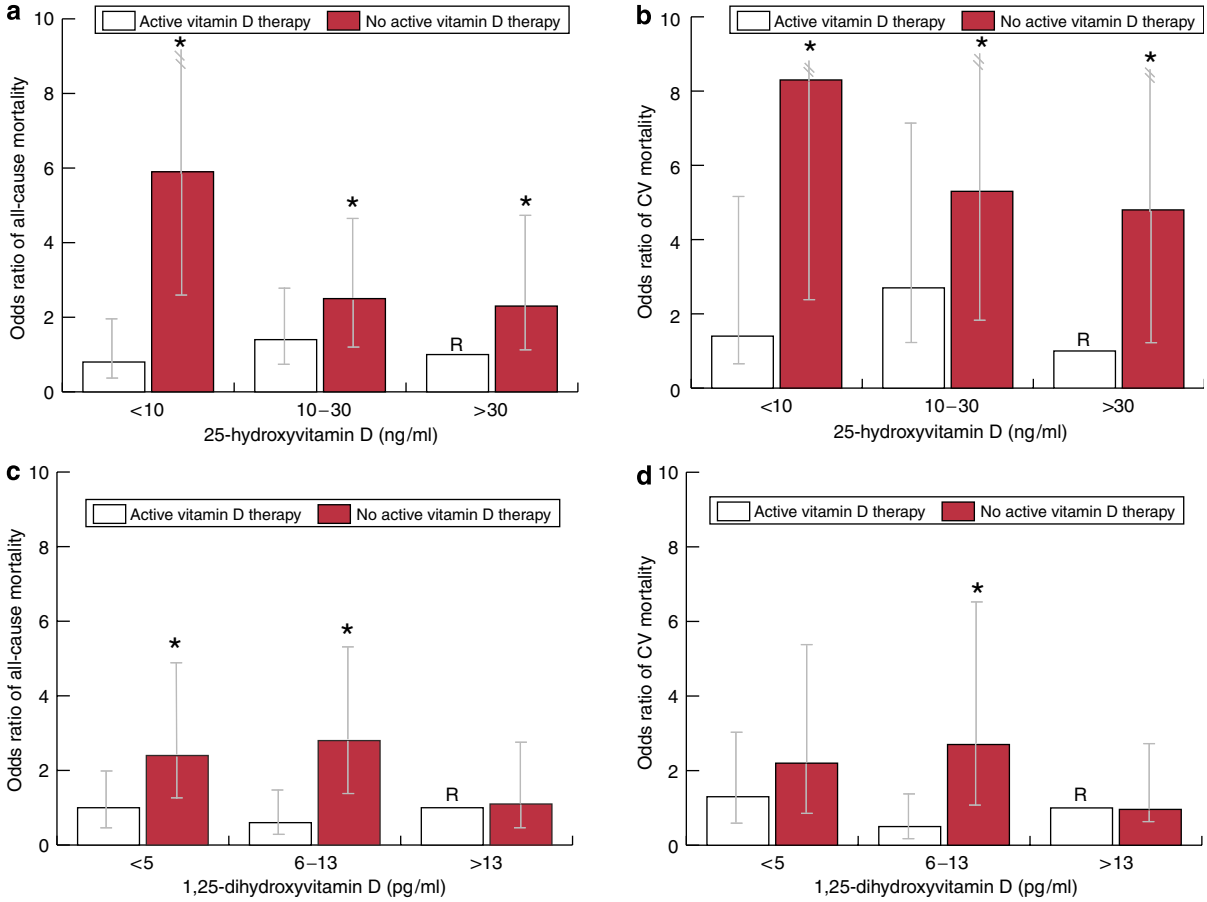

Figure 3 | Multivariate-adjusted ORs of 90-day all-cause and cardiovascular (CV) mortality on hemodialysis according to vitamin D levels and whether or not patients received treatment with active vitamin D. (**a**) 25-hydroxyvitamin D and all-cause mortality; (**b**) 25-hydroxyvitamin D and CV mortality; (**c**) 1,25-dihydroxyvitamin D and all-cause mortality; (**d**) 1,25-dihydroxyvitamin D and CV mortality. The reference groups (R) were subjects who were treated with active vitamin D and had 25-hydroxyvitamin D levels ⩾30 ng/ml or 1,25-dihydroxyvitamin D levels ⩾13 pg/ml. $N=984$ for 25-hydroxyvitamin D analyses and 719 for 1,25-dihydroxyvitamin D analyses; *$P<0.05$ for the comparison of the individual vitamin D level—vitamin D treatment groups with the corresponding referent groups.

($P<0.01$ for 25D × active vitamin D; $P=0.01$ for 1,25D × active vitamin D). Therefore, additional analyses were performed with vitamin D levels, and active vitamin D treatment interaction categories included in the model. Figure 3 illustrates the risk of all-cause and cardiovascular mortality within the groups categorized by vitamin D levels and active vitamin D therapy (summarized as T+, received therapy or T−, untreated). In these multivariate-adjusted analyses, the reference groups were T+ subjects in the highest vitamin D groups (25D >30 ng/ml; 1,25D >13 pg/ml). Other covariates included in the multivariate models included age, sex, race, etiology of real failure, standardized mortality rates, blood pressure, vascular access, albumin, creatinine, PTH, calcium, phosphorus, hemoglobin, and a past medical history of coronary artery disease, stroke, malignancy, or congestive heart failure. Compared to the 25D referent group, there was a monotonic increase in risk of all-cause mortality among T− subjects with decreasing 25D

levels (Figure 3a): baseline 25D levels >30 ng/ml (OR 2.3; 95% CI 1.1, 5.1), 10–30 ng/ml (OR 2.5; 95% CI 1.3, 5.0), and <10 ng/ml (OR 5.9; 95% CI 2.6, 13.7). The association between decreasing 25D levels and increased risk among T− subjects increased when restricted to cardiovascular mortality (Figure 3b): 25D levels >30 ng/ml (OR 4.8; 95% CI 1.5, 15.0), 10–30 ng/ml (OR 5.3; 95% CI 1.8, 15.4), and <10 ng/ml (OR 8.3; 95% CI 2.4, 28.7). Although the effect size was not monotonic and less strong compared to the 25D analyses, T− patients with 1,25D levels 6–13 pg/ml (OR 4.1; 95% CI 2.0, 8.2) and <6 pg/ml (OR 2.1; 95% CI 1.0, 4.6) were also at significantly increased risk of early all-cause mortality compared to the 1,25D referent group (Figure 3c); T− patients with 1,25D levels between 6 and 13 pg/ml (OR 4.5; 95% CI 1.9, 10.4) were at significantly increased risk of early cardiovascular mortality (Figure 3d). In all of these analyses, there was no increase in risk of mortality according to 25D or 1,25D levels in the subjects who subsequently received active vitamin D therapy (Figure 3a–d).

In stratified analyses by vitamin D therapy, there was no association between 25D, 1,25D, and all-cause mortality among subjects subsequently treated with active vitamin D. In an analysis of 25D restricted to T− subjects ($n=324$) and parsimoniously adjusted for age, sex, PTH, and albumin, the OR of all-cause mortality was 1.0 (95% CI 0.6, 1.8; $P=0.9$) for 25D levels of 10–30 ng/ml and 2.2 (95% CI 1.0, 5.1; $P=0.05$) for 25D levels <10 ng/ml compared with 25D levels >30 ng/ml. In a similar stratified model of 1,25D ($n=237$), the adjusted OR of all-cause mortality was 3.1 (95% CI 1.5, 6.4; $P<0.01$) for 1,25D levels of 6–13 pg/ml and 1.5 (95% CI 0.7, 3.3; $P=0.3$) for 1,25D levels <6 pg/ml compared with 1,25D levels >13 pg/ml.

DISCUSSION

Incident hemodialysis patients living throughout the United States are profoundly deficient in both 25D and 1,25D. Although serum levels of calcium, phosphorus, and PTH are biologically linked with vitamin D metabolism and are traditionally used to identify vitamin D deficiency,[10] these parameters correlated poorly with vitamin D levels. Lower serum levels of both 25D and 1,25D were associated with increased mortality within 90 days of initiating hemodialysis, a finding that was independent of standard nutritional factors, residual renal function, comorbidities, other known predictors of mortality on dialysis, and importantly, biomarkers of mineral metabolism. To our knowledge, this is the largest study of incident hemodialysis patients throughout the United States to examine 25D and 1,25D levels, and to determine the relationships between these levels, traditional biomarkers of mineral metabolism, and mortality.

The prevalence of vitamin D deficiency ranges between 20 and 50% in the general population.[2,4,10] This high variability likely reflects differences in the racial distribution and sunlight exposure in the populations that were studied, along with their prevalence of obesity, diabetes, advanced age, nephrotic syndrome, and malabsorption, all risk factors for vitamin D deficiency. Differences in laboratory measures may have also contributed to the variability. In this study in which all samples were tested in a single laboratory and during the same time period, 78% of new hemodialysis patients were vitamin D deficient. Although this high prevalence may relate in part to the large representation of black and diabetic subjects, which is typical of US dialysis populations, these factors are unlikely to fully account for the pervasiveness and severity of 25D deficiency we observed. While this is especially noteworthy, as 25D is synthesized by the liver and does not depend on normal renal function,[36] the cutaneous production of cholecalciferol, the precursor of 25D, is impaired in uremia.[37] In contrast, circulating 1,25D levels largely depend on the ability of the kidney's 1α-hydroxylase to convert 25D to 1,25D. Severe 1,25D deficiency in patients with renal failure is often assumed, but this is the first study to document it in a large incident hemodialysis cohort and to demonstrate its association with mortality.

It is interesting that the magnitude of risk of mortality was significantly greater for decreased 25D levels than decreased 1,25D levels. This may reflect preferable characteristics of 25D as a biomarker compared with 1,25D, including its more reproducible assay, longer half-life and thus more accurate measure of exposure over time, and lack of regulation within a narrow range.[2] Differences in the reference groups may also explain the discrepancy. The reference group for the 25D analyses was made up of patients with normal levels against whom deficient patients were compared, whereas the reference group for the 1,25D analyses included mostly patients with severely reduced 1,25D levels. In addition to these study design issues, it is also possible that 25D has important biological effects, such as on the cardiovascular system, even in dialysis patients.[38] Although 1,25D has greater affinity for the vitamin D receptor, many cells also express 1α-hydroxylase, including vascular smooth muscle and endothelial cells.[39–41] 1,25D synthesized from circulating 25D in these cells can bind local receptors in autocrine/paracrine pathways. Importantly, 25D circulates at ~1000-fold higher concentrations than 1,25D, suggesting that maintaining adequate 25D stores may also be necessary, especially for cells that rely on autocrine pathways.

Although previous observational studies suggest a significant survival advantage associated with activated vitamin D therapy,[29–32,34] selection bias and residual confounding may have contributed to the results. A critical strength of this study is that clinicians did not have baseline 25D or 1,25D levels to influence their management decisions. Therefore, even if 'healthier' patients were more likely to be selected for treatment with active vitamin D, such an argument cannot explain the observation that lower levels of 25D and 1,25D were associated with an increased risk for early mortality, specifically among the patients who never received active vitamin D therapy. It is possible that the sickest patients had the lowest vitamin D levels, and that the increased mortality risk was due to antecedent illness rather than vitamin D

deficiency itself. Nevertheless, the graded effect we observed was independent of comorbidities and other indicators of overall health such as albumin and creatinine levels. Furthermore, uncovering a statistically significant interaction between endogenous vitamin D levels, subsequent vitamin D therapy, and survival—namely, an increased risk of death specifically among those deficient patients who were not subsequently treated with active vitamin D—lends further biological support to the significance of low vitamin D levels. It must be emphasized, however, that only a randomized controlled trial could provide definitive proof of a benefit of treating vitamin D-deficient dialysis patients with vitamin D, activated vitamin D, or both.

Vitamin D measurements are not part of routine dialysis management, and only recently they have been recommended in predialysis kidney disease patients.[42] Even in the latter group, however, the decision to measure 25D is often linked to management of secondary hyperparathyroidism, which is but one of several important biological targets for vitamin D. Non-dialysis patients demonstrate a strong inverse relationship between 25D levels below $\sim$30 ng/ml and PTH,[1,10] and small dialysis studies have also reported an inverse relationship between PTH and 25D levels, with correlations ranging from -0.23 to -0.32.[25,43–45] In this study of nearly 1000 patients from throughout the United States, blood levels of PTH were a poor surrogate of 25D or 1,25D levels. Indeed, almost 80% of patients with serum PTH levels $<$150 pg/ml were nonetheless vitamin D deficient. Thus, relying on PTH to define whom to treat, rather than directly measuring vitamin D levels, precludes the possibility of identifying vitamin D deficiency in many patients who may be at dramatically increased risk for early mortality.

Although these results suggest that perhaps patients with kidney disease should be screened for vitamin D deficiency, we cannot recommend routine measurements of vitamin D levels in the clinical management of dialysis patients as a result of this single study. We must exercise caution because only a placebo-controlled, randomized trial of therapy could prove a causal relationship between vitamin D levels, therapy, and survival. Indeed, the nephrology literature is replete with examples where strong epidemiological data support an increased mortality risk associated with a deficiency (or excess) of a biomarker, yet direct interventions to correct the abnormality did not alter outcomes when tested in a randomized trial.[46–48] In addition, the relatively short follow-up of incident patients we studied limits our ability to generalize the results to prevalent patients over longer durations of follow-up and increases the possibility of confounding by comorbidities present before initiation of dialysis. Although we adjusted for differences in comorbidities, residual confounding remains a limitation. Therefore, our results must be verified by other investigators in different cohorts. If the results were consistent, future interventional studies could be justified, particularly among patients who initiate hemodialysis with PTH levels below the current threshold for initiating vitamin D therapy.

MATERIALS AND METHODS

Accelerated Mortality on Renal Replacement (ArMORR) is a nationally representative prospective cohort study of patients who initiate chronic hemodialysis at any one of $>$1000 US dialysis centers operated by Fresenius Medical Care, North America. ArMORR contains detailed demographic and clinical data, including comorbidities, laboratory results, and blood samples at dialysis initiation and every 90 days thereafter to 1 year. Clinical data, including results of laboratory tests and records of drug administration, are collected prospectively and entered uniformly into a central database by practitioners at the point of care. All clinical data points arriving at Fresenius undergo rigorous quality assurance/quality control (QA/QC) auditing because these data are directly linked to Medicare billing services, and routine QA/QC measures are mandated by the Clinical Quality Group and Data Entry Error Reduction Task Force at Fresenius.[32,33] All blood samples collected for clinical care are uniformly shipped to and processed by Spectra East (Rockland, NJ, USA), a good clinical practice (GCP)-accredited central laboratory. After processing for routine clinical testing, residual samples are shipped on ice to the ArMORR investigators where the samples are aliquoted and stored in liquid nitrogen tanks. This study was approved by the Institutional Review Board of the Massachusetts General Hospital, which waived the need for informed consent.

Study population

Between 1 July 2004 and 30 June 2005, 10 044 incident hemodialysis patients representing 1056 US dialysis units were prospectively enrolled into ArMORR. Of these 10 044 patients, 19 (0.2%) had levels of either 25D or 1,25D measured for routine clinical purposes within 90 days of initiating hemodialysis, whereas virtually all patients had repeated measurements of calcium, phosphorus, and PTH during the same period.

For this study, we excluded subjects who had initiated therapy with oral or injectable vitamin D therapy before the collection of their baseline blood sample. The first 825 consecutive patients who enrolled in ArMORR and met these inclusion criteria were used to study the distribution of baseline vitamin D levels and their association with other markers of mineral metabolism in a representative incident hemodialysis cohort. Of these 825 subjects, 75 (9%) died within 90 days of initiating dialysis and 750 survived for at least 90 days. To efficiently study the effects of 25D and 1,25D levels on survival, we performed a nested case–control study defining cases as subjects who died within 90 days of initiating dialysis and controls as those who survived for at least 90 days. To increase power, we added the next 175 consecutive ArMORR participants who died within 90 days of initiating dialysis ($n = 250$ total cases) to the original sample to create a case–control sample of 1:3 ratio with a total of 1000 subjects. All cases were members of the 10 044 subjects recruited for ArMORR between 1 July 2004 and 30 June 2005; no additional subjects were recruited for this study after the year of ArMORR enrollment. With a case–control sample of 1000 and a 1:3 ratio, we had $>$90% power to detect an OR of 2 among patients with vitamin D deficiency compared to those with normal levels. Of the 1000 subjects, 984 had adequate serum samples for 25D assays (244 cases and 740 controls), and 703 had adequate samples for 1,25D assays (183 cases and 527 controls). There were no significant differences in demographic or baseline laboratory characteristics among those with inadequate samples compared with those who had sufficient sample volume for both assays.

Exposures, outcomes, and covariates

The primary exposures were baseline circulating levels of 25D and 1,25D that were collected within 14 days of initiating hemodialysis and before starting any oral or injectable vitamin D. After collection, blood samples were frozen in liquid nitrogen and underwent a single thaw for this study following an average storage time of 8 months. Given the number of samples to be tested, 25D and 1,25D levels were measured in duplicate using commercially available radioimmunoassay techniques (DiaSorin Inc., Stillwater, MN, USA).[49–52] The coefficients of variation (CVs) for 25D measurements were $<3\%$ at levels <30 ng/ml and for 1,25D the CVs were $<6.5\%$ at levels <32.5 pg/ml. Based on clinical definitions[2,36] and for purposes of interpretability, 25D levels $\geqslant 30$ ng/ml were considered replete, whereas vitamin D deficiency was defined as a level <30 ng/ml and severe deficiency as levels <10 ng/ml. As cut points for 1,25D levels are less well defined, we analyzed tertiles of 1,25D levels according to its distribution in the overall population. Blood levels of PTH were measured using the Nichols Advantage Bio-Intact PTH assay that detects bioactive (1–84) hormone.

The primary outcome was all-cause mortality within 90 days after initiating dialysis, and the secondary outcome was cardiovascular mortality. Ninety-day mortality was chosen as the primary end point for several reasons. The rate of early mortality on hemodialysis is almost double that seen after the first 90 days,[53] yet risk factors for early mortality have been mostly understudied. In addition, outcome events during this time period are proximate to the primary exposure and thus are affected less by co-interventions and other potential confounders. Finally, active vitamin D use increases over time on hemodialysis, a phenomenon that would limit the statistical power of analyses of the effects on survival of vitamin D levels among untreated patients. Death was confirmed by mandatory discharge diagnosis reports from dialysis centers, and cardiovascular deaths were determined based on International Classification of Diseases (ICD)-9 mortality codes, despite their limitations, as done previously.[32,33]

We analyzed several covariates including age, gender, race, etiology of renal failure, blood pressure, body mass index, dialysis access at initiation (arteriovenous fistula, graft, or venovenous catheter), dialysis dose assessed by the urea reduction ratio, facility-specific standardized mortality rates,[54] and comorbidities at the initiation of dialysis (diabetes, hypertension, coronary artery disease, myocardial infarction, peripheral vascular disease, stroke, congestive heart failure, chronic obstructive pulmonary disease, non-cutaneous malignancy, and liver disease). Comorbidities were ascertained by the individual patients' practitioners and derived from the initial intake history, physical examination, and medical records such as referrals and discharge summaries. To further capture overall health status, we also examined whether patients initiated hemodialysis as an outpatient vs in the hospital. Finally, we analyzed the first results of laboratory tests obtained at the initiation of dialysis including sodium, potassium, bicarbonate, blood urea nitrogen, creatinine, calcium, phosphorus, PTH, alkaline phosphatase, albumin, and hemoglobin.

Analysis

We used two-sample *t*-tests and Fisher's exact test to compare demographic and laboratory characteristics and vitamin D levels at the initiation of dialysis among the patients who died and the 750 patients who survived. To examine whether routine laboratory tests of mineral metabolism were associated with decreased vitamin D levels, we used Spearman correlation coefficients to summarize the correlations between vitamin D levels and biomarkers of mineral metabolism. We used linear regression models to examine trends in mean levels of mineral metabolites across the vitamin D groups.[55] In addition, as PTH is currently the clinical standard of care used to define when to administer active vitamin D treatment, we examined the range of 25D and 1,25D levels across the spectrum of PTH measurements and tested differences in vitamin D levels according to PTH levels $\leqslant$ or >150 pg/ml.

We used logistic regression analyses on the case–control sample of 1000 patients to examine survival on hemodialysis according to baseline vitamin D levels. We performed separate analyses for 25D and 1,25D, examining them as continuous and also as categorical exposures using the cut points described above to test for potential nonlinear associations. Although we excluded subjects who received vitamin D therapy before the measurement of their vitamin D levels to limit misclassification of exposure, 62% of subjects subsequently initiated therapy with active vitamin D during the follow-up period, which is consistent with national data.[29,32] In active vitamin D-treated subjects, therapy began at a median of 14 days after initiation of dialysis and continued for a median duration of 74 days. Independent predictors of subsequent therapy with active vitamin D included baseline PTH (higher levels associated with therapy) and phosphorus levels (lower levels associated with therapy). As exogenous vitamin D therapy could offset risk associated with decreased endogenous vitamin D levels, we tested for interaction[55] between vitamin D levels and survival by active vitamin D therapy using χ^2 tests derived from logistic regression models. When significant ($P<0.05$) interaction was detected, we analyzed multivariate-adjusted logistic regression models with interaction terms (3 levels of 25D and 1,25D $\times$ active vitamin D treatment status) and models stratified by vitamin D therapy. We also tested for interaction between vitamin D levels and PTH.

Multivariate logistic regression models were used to adjust for potential confounders. We included covariates in the multivariate models that have been associated with mortality on dialysis in previous studies and those that were significantly different among cases and controls in this study. Data points on individual covariates were missing in $<5\%$ of subjects; for the multivariate analyses, these covariates were treated as categorical variables with an additional category for missing values. Otherwise, continuous variables were analyzed on a continuous scale. PTH was also examined as a binary variable ($\leqslant$ or >150 pg/ml).

As vitamin D levels are influenced by climate and season, we also adjusted for the season (summer: 1 April to 30 September vs winter: 1 October to 31 March) and the states (divided into four groups according to latitude) in which patients initiated dialysis, as performed in previous vitamin D studies.[2,10,56] Finally, we measured vitamin D-binding protein, the primary binding protein for circulating vitamin D, in a randomly selected subset of 100 cases and 200 controls using an automated immunonephelometric method (Dade Behring BNII, Marburg, Germany) with CVs $<7\%$, which permitted analyses of the association between levels of vitamin D and mortality independent of its carrier protein. Adjusting models for vitamin D-binding protein levels, coding for missing values, did not alter the results (data not shown). All results are reported as mean $\pm$ s.d. Analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

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DISCLOSURE

M Wolf has received honoraria from Abbott Laboratories, Genzyme, and Shire. R Thadhani has received research support from Abbott Laboratories and honoraria from Abbott Laboratories and Genzyme. Drs Wolf and Thadhani have a patent filed by the Massachusetts General Hospital linking Vitamin D deficiency with dialysis mortality.

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