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Original Investigation

Biomarkers of Vitamin D Status and Risk of ESRD

Casey M. Rebholz, PhD, MS, MPH,[1] Morgan E. Grams, MD, PhD, MHS,[1,2] Pamela L. Lutsey, PhD, MPH,[3] Andrew N. Hoofnagle, MD, PhD,[4] Jeffrey R. Misialek, MPH,[3] Lesley A. Inker, MD, MS,[5] Andrew S. Levey, MD,[5] Elizabeth Selvin, PhD, MPH,[1,6] Chi-yuan Hsu, MD, MSc,[7] Paul L. Kimmel, MD,[8] Ramachandran S. Vasan, MD,[9,10] John H. Eckfeldt, MD, PhD,[11] and Josef Coresh, MD, PhD, MHS,[1,6] on behalf of the Chronic Kidney Disease Biomarkers Consortium*

Background: Disordered mineral metabolism is characteristic of decreased kidney function. However, the prospective associations between circulating levels of vitamin D binding protein, vitamin D, and end-stage renal disease (ESRD) have not been extensively evaluated in epidemiologic studies.

Study Design: Nested case-control study.

Setting & Participants: Middle-aged black and white men and women from 4 US communities.

Predictors: Baseline levels of vitamin D binding protein, 25-hydroxyvitamin D (25[OH]D), and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) were measured in blood samples collected at study visit 4 (1996-1998) of the ARIC (Atherosclerosis Risk in Communities) Study.

Outcome: ESRD cases (n = 184) were identified through hospitalization diagnostic codes from 1996 to 2008 and were frequency matched to controls (n = 251) on categories of estimated glomerular filtration rate, albuminuria, diabetes mellitus, sex, and race.

Measurements: Logistic regression was used to estimate the association between mineral metabolism biomarkers (vitamin D binding protein, 25(OH)D, and $1,25(OH)_2D$) and incident ESRD, adjusting for age, sex, race, estimated glomerular filtration rate, albuminuria, diabetes mellitus, hypertension, education, specimen type, and serum levels of calcium, phosphate, and parathyroid hormone.

Results: Higher vitamin D binding protein levels were associated with elevated risk for incident ESRD (OR, 1.76; 95% CI, 1.22-2.54; $P = 0.003$). Higher free and bioavailable 25(OH)D levels were associated with reduced risk for incident ESRD (ORs of 0.65 [95% CI, 0.46-0.92; $P = 0.02$] and 0.63 [95% CI, 0.43-0.91; $P = 0.02$] for free and bioavailable 25[OH]D, respectively). There was no association between ESRD and overall levels of 25(OH)D (OR, 0.83; 95% CI, 0.58-1.19; $P = 0.3$) or $1,25(OH)_2D$ (OR, 0.73; 95% CI, 0.48-1.13; $P = 0.2$).

Limitations: Lack of direct measurement of free and bioavailable vitamin D.

Conclusions: In the general population, blood levels of vitamin D binding protein were positively associated and blood levels of free and bioavailable 25(OH)D were inversely associated with new-onset ESRD during follow-up.

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INDEX WORDS: Biological markers; chronic renal failure; end-stage renal disease (ESRD); risk factors; vitamin D-binding protein; vitamin D; mineral metabolism biomarker; vitamin D insufficiency.

From the [1]Department of Epidemiology and Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Bloomberg School of Public Health; [2]Division of Nephrology, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD; [3]Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN; [4]Department of Laboratory Medicine, University of Washington, Seattle, WA; [5]William B. Schwartz Division of Nephrology, Department of Medicine, Tufts Medical Center, Boston, MA; [6]Division of General Internal Medicine, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD; [7]Division of Nephrology, Department of Medicine, University of California, San Francisco School of Medicine, San Francisco, CA; [8]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; Sections of [9]Preventive Medicine and Epidemiology and [10]Cardiology, Department of Medicine, Boston University School of Medicine, Boston, MA; and [11]Department of Laboratory Medicine and Pathology, University of Minnesota School of Medicine, Minneapolis, MN.

**A full list of Chronic Kidney Disease Biomarkers Consortium Investigators is available at www.ckdbiomarkersconsortium.org.*

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Address correspondence to Casey M. Rebholz, PhD, MS, MPH, Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, Welch Center for Prevention, Epidemiology, and Clinical Research, 2024 E Monument St, Ste 2-600, Baltimore, MD 21287. E-mail: crebhol1@jhu.edu

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Disordered mineral metabolism is one of the earliest complications of chronic kidney disease (CKD).[1-3] Decreased glomerular filtration rate (GFR) is associated with lower 1α-hydroxylase activity, which results in decreased activation of 25-hydroxyvitamin D (25[OH]D) to 1,25-dihydroxyvitamin D (1,25 $[OH]_2D$), leading to hypocalcemia and hyperparathyroidism.[1,4-6] Adequate vitamin D levels are a therapeutic goal for patients with kidney disease.[7] However, clinical recommendation to use vitamin D supplementation in patients with CKD for correcting vitamin D deficiency is opinion based and needs additional empirical evidence.[8] Detecting deficient vitamin D levels may be useful in estimating future risk for kidney disease progression.[2,9,10]

In clinical and research settings, 25(OH)D levels are usually reported as an assessment of vitamin D status. The majority (approximately 85%-90%) of circulating 25(OH)D, like $1,25(OH)_2D$, is tightly bound to vitamin D binding protein and is thought to be biologically inactive.[11] Bioavailable 25(OH)D consists of a smaller amount (10%-15%) that is loosely bound to albumin and <1% that is circulating in a free and unbound form.[11-13] Binding affinities for 25(OH)D vary by isoforms of vitamin D binding protein; these isoforms differ by racial group, and they explain some of the variability in circulating levels of vitamin D binding protein and 25(OH)D.[14-16] Compared with previous studies of 25(OH)D, current efforts can now more completely evaluate the association of 25(OH)D with adverse outcomes using blood levels and isoforms of vitamin D binding protein to estimate free and bioavailable 25(OH)D.

The objective of this study was to assess the relationship between vitamin D–related biomarkers with incident end-stage renal disease (ESRD) in a community-based population, the ARIC (Atherosclerosis Risk in Communities) Study, in collaboration with the Chronic Kidney Disease Biomarkers Consortium. We hypothesized that blood levels of vitamin D binding protein would be positively and independently associated with ESRD risk after accounting for demographics, kidney measures, and known kidney disease risk factors. Furthermore, we hypothesized that blood levels of 25(OH)D and, secondarily, alternative measures of vitamin D status, including $1,25(OH)_2D$, free vitamin D, and bioavailable vitamin D, would be inversely and independently associated with ESRD risk. We explored the distribution of C3-epimer of 25-hydroxyvitamin D_3 (3-*epi*-$25(OH)D_3$), a form of vitamin D that is not well characterized, according to incident ESRD case status.[17]

METHODS

Study Design

The ARIC Study is a prospective cohort study of 15,792 predominantly black and white men and women aged 45 to 64 years at enrollment from 4 US communities: Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD. ARIC Study participants were recruited and enrolled in 1987 to 1989, and 4 follow-up study visits were conducted: 1990 to 1992, 1993 to 1995, 1996 to 1998, and 2011 to 2013. The ARIC Study is described in detail elsewhere.[18] In the present nested case-control study, ARIC Study visit 4 (1996-1998) was the baseline visit. The main reason for using study visit 4 as baseline for the present analysis was that urinary albumin-creatinine ratio (UACR) was measured at this time point, which is an important indicator of kidney damage.

Study Participants

A total of 11,656 ARIC Study participants (73.8% of the original ARIC cohort) completed the baseline (visit 4) examination. Study participants were excluded from the present nested case-control study if they were missing information on the factors making up the frequency-matching categories (estimated GFR [eGFR], UACR, diabetes mellitus, sex, and race) or developed ESRD prior to baseline. Incident ESRD cases (n = 184) were defined using diagnostic codes for hospitalizations and deaths identified through active surveillance from baseline (1996-1998) through December 31, 2008. ESRD case status was defined by: (1) *International Classification of Diseases* (*ICD*) codes for hospitalizations related to kidney transplantation, dialysis, or procedural code indicating dialysis, excluding hospitalizations with concomitant *ICD* codes for traumatic anuria (958.5) or acute kidney injury (586 and 788.9); or (2) death certificates with kidney failure–related *ICD* codes (584-584.9, 586, and N17.0) as an underlying cause of death and history of CKD. This outcome definition is described in detail elsewhere.[19] Frequency matching was used to identify controls (n = 251) based on eGFR category (<45, 45-59, 60-74, 75-89, 90-104, and ≥105 mL/min/1.73 m^2), UACR category (<30, 30-300, and >300 mg/g), diabetes mellitus, sex, and race. Controls were selected to match the frequency of cases within each stratum (approximately 1-2 controls per case within each stratum). These strong risk factors for ESRD (diabetes mellitus, sex, and race) and indicators of kidney function (eGFR) and kidney damage (UACR) were selected as matching factors in order to evaluate the ability of novel biomarkers to predict ESRD risk beyond established factors. The study protocol was approved by the institutional review boards of all participating institutions, and written documentation of informed consent was obtained from all study participants.

Measurement of Covariates

At visit 4 (baseline for the present study), a questionnaire was administered, blood pressure and anthropometrics were measured, and blood specimens and spot urine specimens were collected. The questionnaire was administered by trained staff to assess demographic characteristics (age, sex, and race), socioeconomic status (education level), health behaviors, medical history, and medication use. Participants brought current medications to the study visit, and medications were transcribed and coded. Blood pressure measurements were taken by a certified technician using a random-zero sphygmomanometer after the participant was seated and resting for 5 minutes. Body mass index was calculated as weight (in kilograms) divided by the square of height (in meters) from measurements taken while participants wore light clothing without shoes. Glucose was measured by the modified hexokinase/glucose-6-phosphate dehydrogenase method. Creatinine was measured in plasma and urine by the modified kinetic Jaffé method, and values were calibrated to the National Institute of Standards and Technology standard. Albumin was measured in urine specimens by a nephelometric method on a Dade Behring BN100 and Beckman Image Nephelometer. Serum calcium (coefficient of variation [CV], 1.3%) and phosphorus (CV,