Serum phosphorus and association with anemia among a large diverse population with and without chronic kidney disease

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ABSTRACT

Background. We hypothesized that phosphorus has an effect on anemia in both normal kidney function and early chronic kidney disease (CKD). We sought to determine whether higher phosphorus levels are associated with anemia in a large diverse population without CKD and early CKD.

Methods. This study is a historical population-based study within the Kaiser Permanente Southern California health system (1 January 1998 to 31 December 2013) among individuals aged 18 years and older with estimated glomerular filtration rate >30 mL/min/1.73 m^2 and measurements of serum phosphorus, creatinine and hemoglobin. Individuals were excluded if they had secondary causes of anemia. Odds ratio (OR) estimated for moderate anemia defined as hemoglobin <11 g/dL for both sexes. Mild anemia was defined as <12 g/dL (females) and <13 g/dL (males).

Results. Among 155 974 individuals, 4.1% had moderate anemia and 12.9% had mild anemia. Serum phosphorus levels ≥3.5 mg/dL were associated with both mild and moderate anemia. Moderate anemia OR (95% confidence interval) was 1.16 (1.04–1.29) for every 0.5 mg/dL phosphorus increase and 1.26 (1.07–1.48) in the highest versus middle phosphorus tertile. Additional independent anemia risk factors, including female sex, Asian race, diabetes, low albumin and low iron saturation, were observed, but did not alter the anemia–phosphorus association.

Conclusions. Higher phosphorus levels were associated with a greater likelihood for anemia in a population with early CKD and normal kidney function. Phosphorus may be a biomarker for anemia and may affect aspects of hematopoiesis.

Keywords: anemia risk, epidemiology, serum phosphorus

INTRODUCTION

Phosphorus plays a major role in physiological functioning, including energy production, cellular replication and bone mineral metabolism. The level of phosphorus is tightly regulated by three main hormones [parathyroid hormone (PTH), vitamin D and fibroblast growth factor-23 (FGF-23)], which affect the intestinal absorption and renal excretion of phosphorus and bone mineral metabolism [1]. Dysregulation of these processes resulting in chronically low or high serum phosphorus has been associated with adverse outcomes [2]. Although chronic kidney disease (CKD) often leads to hyperphosphatemia, abnormalities in phosphorus levels have been observed in populations with and without kidney disease.

Hyperphosphatemia is associated with inflammation and may affect normal cellular physiology including erythropoiesis [3, 4]. Phosphorus alone has been implicated in inhibiting red blood cell production in uremic patients [5]. In dialysis and kidney transplant patients, hyperphosphatemia is associated with anemia independent of other mineral bone disease components [6, 7]. Along with anemia, hyperphosphatemia has been linked with lower bone mineral density in the peritoneal dialysis population [5]. It is also associated with high levels of PTH, which has been shown to inhibit erythropoiesis, induce hemolysis and cause bone marrow fibrosis in CKD states [8–12]. In addition, FGF-23 and its interplay with klotho, vitamin D and iron have been implicated with anemia [13–15]. In murine models that lack FGF-23 or klotho, the resultant hyperphosphatemia is associated with cell toxicity, premature aging and vascular calcifications [16].

Determining a relationship between phosphorus levels and prevalence of anemia in the non-CKD population may have important clinical implications. A study evaluating the National

Health and Nutrition Examination Survey population showed that serum phosphorus levels >4.4 mg/dL were associated with mild anemia among individuals with normal kidney function [estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2] [17]. Another study of kidney transplant patients showed that a one standard deviation higher of serum phosphorous level (0.8 mg/dL) was associated with 77% greater odds for anemia [7]. These few studies that evaluated the relationship between serum phosphorus and anemia have been limited in various ways, including smaller population size, lack of heterogeneity and non-consideration of secondary causes of anemia [7, 17].

Given the biological importance of phosphorus, a better understanding of its levels in the body and association with pathophysiological processes such as anemia would provide invaluable insights. The objective of our study was to determine whether higher phosphorus levels were associated with anemia in a large diverse population with and without early CKD. We hypothesized that higher phosphorus levels increase the likelihood for anemia in people with both normal kidney function and mild CKD.

MATERIALS AND METHODS

Study design and setting

A historical population cohort study of Kaiser Permanente Southern California (KPSC) members was performed in the period of 1 January 1998 through 31 December 2013. The KPSC health system is an integrated healthcare system comprising 14 medical centers and over 200 satellite clinics, geographically spanning from Bakersfield to San Diego, CA, USA. As of 31 December 2013, there were over 3.6 million members of whom more than 2.4 million were adults. The membership population is racially and ethnically diverse reflective of the practicing area and the state of California [18]. All members have similar access to healthcare facilities, procedures and referrals. All information collected during clinical care is captured in a common electronic health record system. The study was approved by the regional Institutional Review Board and exempted from informed consent.

Inclusion and exclusion criteria

The study population included individuals aged 18 years and older with continuous membership within KPSC for at least 6 months to ensure documentation of comorbidities. Individuals must have had a minimum of one serum phosphorus, serum creatinine and hemoglobin measurement within 120 days of phosphorus measurement. The first available serum phosphorous value was used as the index date, and individuals with an eGFR >30 mL/min/1.73 m^2 within 120 days of phosphorus measurement were included in the study.

Individuals were excluded if they had serum phosphorus levels at the highest 0.5% or lowest 0.5% to eliminate outliers and potential assay interactions. To eliminate confounders for anemia, individuals were excluded if they were receiving hemodialysis, erythrocyte-stimulating agents or blood transfusions or had other secondary causes of anemia including any organ transplant, malignancy, hemorrhage, hematological disorder, pregnancy, inflammatory or autoimmune disorder or diseases of the liver, lung or spleen. The secondary causes of anemia were determined by inpatient and outpatient International Classification of Diseases (ICD-9 ninth revision) diagnoses coding (Supplementary data, Table S1).

Data sources and laboratory measurements

Data on age, sex, race/ethnicity, laboratory values and comorbidities were extracted from the electronic health records. Race/ethnicity was categorized as white, black, Hispanic, Asian or other. Individuals were categorized as other if no race data were available or if they were not classified as any of the other races. Comorbidities, including diabetes mellitus, were assessed on the basis of inpatient and outpatient ICD-9 coding. The earliest serum creatinine within 120 days after baseline phosphorus measurement was used to establish eGFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [19]. Serum phosphorus level was measured using a standard colorimetric method with normal reference values of 2.7–4.5 mg/dL (Roche Diagnostics, Alameda, California). When available, laboratory values on serum albumin, ferritin, iron saturation and total iron binding capacity were extracted. All laboratory measurements were performed and reported from an American College of Pathology/Clinical Laboratory Improvement Act-certified laboratory.

Statistical methods

The primary outcome evaluated was moderate anemia defined by the World Health Organization (WHO) as hemoglobin <11.0 g/dL for both sex groups [20]. Secondary outcome was mild anemia defined by the WHO as hemoglobin <12.0 g/dL for females and <13.0 g/dL for males. Individuals were categorized into population tertiles on the basis of the distribution of serum phosphorus levels, with the middle tertile serving as the reference group (Supplementary data, Figure S1).

Each individual contributed only one cross-sectional phosphorus and hemoglobin combination. The distributions of categorical variables were assessed via χ^2 or Fisher's exact test and continuous variables via independent samples t-test or non-parametric Kruskal–Wallis test, as appropriate. Univariate and multivariable logistic regression analyses were used to estimate the odds ratio (OR) for mild and moderate anemia across population-based phosphorus tertiles and per 0.5 mg/dL increments of phosphorus. Multivariable models included phosphorus as the exposure and potential confounders that included age, sex, race/ethnicity, diabetes mellitus, albumin, iron saturation and ferritin. Missing variable data were not included in the analyses. Restricted cubic spline modeling was employed to estimate continuous ORs across differing serum phosphorus levels. Pearson's correlation coefficients were estimated for hemoglobin and phosphorus measurements within the entire study population and by sex and race/ethnicity.

RESULTS

Cohort characteristics

A total of 360 379 individuals were identified with out-patient phosphorus and hemoglobin measurements. Of these, 204 405 individuals were not eligible on the basis of the exclusion criteria (Figure 1). The study cohort comprised 155 974 individuals. Baseline characteristics of the cohort revealed a mean age of 52 years with 61.6% females, 38.1% whites, 13.6% blacks, 24.6% Hispanics and 6.9% Asians. Diabetes mellitus was present in 15.8% of the population (Table 1).

Anemia outcomes

Moderate anemia. A total of 6410 (4.1%) individuals had moderate anemia (Figure 1). The baseline characteristics for individuals with moderate anemia revealed higher percentages of females, blacks, diabetics, low albumin and low iron saturation compared with those without anemia (Table 1). Serum phosphorus levels ranged from 1.5 to 6.2 mg/dL, with mean phosphorus 3.5 mg/dL in both moderate anemia and non-anemia groups. Mean hemoglobin was 9.9 g/dL and mean eGFR was 80.2 mL/min/1.73 m^2 in the moderate anemia group, compared with hemoglobin 13.9 g/dL and eGFR 85.0 mL/min/1.73 m^2 in the non-anemia group (Table 1). A comparison of different race/ethnic groups showed that blacks had the lowest mean phosphorus (3.5 mg/dL), hemoglobin (13.0 g/dL) and eGFR 77.5 mL/min/1.73 m^2, compared with all other race/ethnic groups (Table 2). Blacks also had the highest percentage of moderate anemia (7.9%).

The population-based phosphorus tertiles ranged as follows: 1.5–3.1 mg/dL (Q1), 3.2–3.6 mg/dL (Q2) and 3.7–6.2 mg/dL (Q3). Individuals in the third tertile had the highest percentage of moderate anemia (Figure 2). Compared with the second tertile, individuals in both the lowest and highest tertiles had a higher OR for moderate anemia in unadjusted models (Table 3). In fully adjusted models, the associations with moderate anemia were attenuated in the lowest tertile, but remained robust in the third tertile with a 26% higher likelihood [OR (95% confidence interval (CI)) 1.26 (1.07–1.48)]. When modeled continuously in a fully adjusted analysis, each 0.5 mg/dL phosphorus elevation was associated with a 16% increased likelihood for moderate anemia [OR (95% CI) 1.16 (1.04–1.29)] (Table 3). In addition, this multivariable adjusted analysis revealed that Asian race 1.53 (1.15–2.01), diabetes 1.17 (1.00–1.37), female sex 1.76 (1.50–2.06) and individuals with low iron saturation 2.57 (2.23–2.98) and low albumin 3.21 (2.67–3.87) were associated with higher likelihood for moderate anemia, whereas each 10 U increase in eGFR was associated with lower moderate anemia likelihood [OR (95% CI) 0.94 (0.91–0.97)] (Table 3 and Figure 3).

A comparison of moderate anemia across various age groups and sex revealed that compared with males aged 18–49, females in all age groups (particularly ages 18–49 and 70+ years) had a higher likelihood for moderate anemia in fully adjusted models. Males in any other age group had no significant increase when compared with the reference group (Supplementary data, Table S3).

Mild anemia. A total of 20 079 (12.9%) individuals had mild anemia (Figure 1). The baseline characteristics of individuals with mild anemia were similar to those with moderate anemia: higher percentages of females, blacks, diabetics, low albumin and low iron saturation (Supplementary data, Table S2). For the mild anemia group, mean serum phosphorus was 3.5 mg/dL,
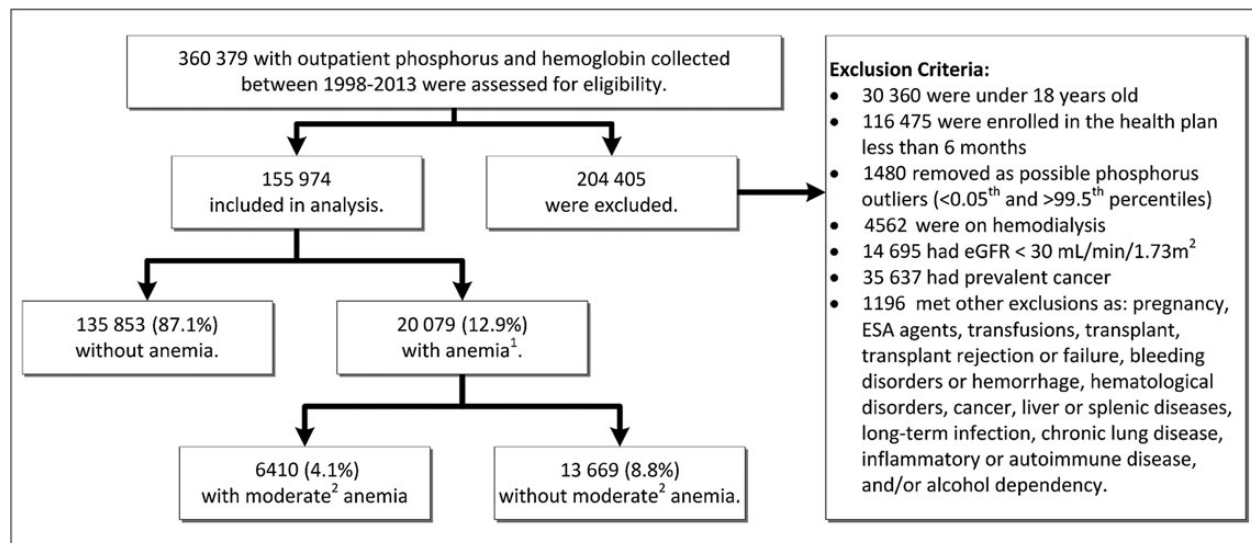
1 – According to WHO definition of mild anemia.
2 – According to WHO definition of moderate anemia.

FIGURE 1: Study cohort flow chart. A total of 360 379 adult KPSC members were identified with outpatient phosphorus and hemoglobin (Hgb) measurements; 204 405 individuals were not eligible on the basis of the exclusion criteria and 155 974 individuals were included in the analysis. Mild anemia, defined as Hgb <12 g/dL for females and <13 g/dL for males, was identified in 12.9% of the cohort (*N* = 20 079). Moderate anemia, defined as Hgb <11 g/dL for both sex, was identified in 4.1% of the cohort (*N* = 6410).

Table 1. Characteristics of study population by moderate anemia status

	No moderate anemia Hgb ≥ 11 g/dL N = 149 564	Moderate anemia Hgb < 11 g/dL N = 6410	Total N = 155 974	P-value
Demographic variables				
Age (years)	52.2 (15.8)	54.3 (17.3)	52.3 (15.8)	<0.001
Female sex (%)	60.9	78.8	61.6	<0.001
Asian race (%)	6.9	7.2	6.9	<0.001
Black race (%)	13.0	25.9	13.6	<0.001
Hispanic race (%)	24.5	27.0	24.6	<0.001
Other (%)	17.2	7.8	16.8	<0.001
White (%)	38.3	32.2	38.1	<0.001
History of diabetes (%)	15.3	26.5	15.8	<0.001
Laboratory parameters				
Serum phosphorus (mg/dL)				
Mean (SD)	3.5 (0.6)	3.5 (0.7)	3.5 (0.6)	<0.001
Median (IQR)	3.5 (3.0, 3.9)	3.5 (3.0, 4.0)	3.5 (3.0, 3.9)	
Hemoglobin (g/dL)				
Mean (SD)	13.9 (1.3)	9.9 (0.9)	13.7 (1.6)	<0.001
Median (IQR)	13.8 (12.9, 14.8)	10.2 (9.5, 10.6)	13.7 (12.7, 14.8)	
eGFR (mL/min/1.73 m^2)				
Mean (SD)	85.0 (23.1)	80.2 (29.4)	84.8 (23.4)	<0.001
Median (IQR)	86.5 (69.0, 102.1)	82.4 (53.9, 105.4)	86.2 (68.6, 10.5)	
Serum albumin (g/dL)				
<4.0	53.7%	85.7%	56.1%	<0.001
≥4.0	46.3%	14.3%	43.9%	
Ferritin (ng/mL)				
<100	49.8%	59.2%	51.2%	<0.001
≥100	50.2%	40.8%	48.8%	
Iron saturation (%)				
<20	34.1	64.5	37.8	<0.001
≥20	65.9	35.5	62.2	

Values are mean (SD) when appropriate. Categorical variables were analyzed using χ^2 or Fisher's exact test, continuous variables using independent samples t-test, Kruskal–Wallis or Mann–Whitney U-test, as appropriate. Higher percentages of females (78.8%), blacks (25.9%), diabetics (26.5%) and individuals with low albumin (85.7%) and low iron saturation (64.5%) were present in the moderate anemia group compared with the non-anemia group.

mean hemoglobin was 11.2 g/dL and mean eGFR was 78.8 mL/min/1.73 m^2 (Supplementary data, Table S2).

A higher percentage of mild anemia was noted in the third tertile (Figure 2). Unadjusted OR for mild anemia demonstrated the highest likelihood in the third tertile and a modestly high likelihood in the lowest tertile when compared with the second tertile (Supplementary data, Table S4). Even after adjusting for demographics, eGFR, albumin, iron saturation and ferritin, the OR for mild anemia was highest in the third tertile 1.35 (1.18–1.55), but no longer significant in the lowest tertile. Moreover, in fully adjusted models of continuous phosphorous levels, each 0.5 mg/dL phosphorus elevation was associated with a 17% higher likelihood for mild anemia [OR (95% CI) 1.17 (1.07–1.28)]. Similar to the moderate anemia group, the multivariable adjusted analysis revealed the following risk factors associated with mild anemia: Asian race 1.35 (1.06–1.72), diabetes 1.47 (1.29–1.67), low iron saturation 2.71 (2.40–3.07) and low albumin 3.12 (2.76–3.57) (Supplementary data, Figure S2). However, in contrast to the moderate anemia group, black race 1.30 (1.11–1.51) and age >70 years 1.48 (1.21–1.77) were significantly associated with higher mild anemia likelihood, and female sex had lower mild anemia OR 0.87 (0.76–0.98). Furthermore, each 10 U (1 U = 1 mL/min/1.73m^2) increase

in eGFR was associated with a 9% lower likelihood for mild anemia [OR (95% CI) 0.91 (0.88–0.93)] (Supplementary data, Table S4).

When comparing the prevalence of mild anemia by age group and sex, a higher percentage of anemia was noted in females aged 18–49 years (Supplementary data, Figure S3). However, after adjusting for demographics, eGFR, serum albumin, ferritin and iron saturation, compared with males aged 18–49 years, the odds of mild anemia were significantly higher in females aged 18–49 and 70+ years, as well as in males aged 50 + years, but highest in males aged 70+ years (Supplementary data, Table S3).

Secondary analyses

Restricted cubic spline modeling of multivariable adjusted OR across various ranges of serum phosphorus revealed a higher likelihood for moderate anemia with phosphorus levels >3.5 and <2.0 mg/dL (Figure 4). For mild anemia, phosphorus levels >3.5 and <2.5 mg/dL were associated with higher likelihood (Supplementary data, Figure S4).

Correlation analyses demonstrated a significant negative correlation between phosphorus and hemoglobin for the entire cohort, among males but not among females, and across all racial/ethnic groups (P < 0.001) (Figure 5).

Table 2. Characteristics of study population categorized by race/ethnicity

	Asian	Black	Hispanic	Other	White	Total	P-value
	n = 10 829 (6.9)	n = 21 159 (13.6)	n = 38 363 (24.6)	n = 26 247 (16.8)	n = 59 376 (38.1)	n = 155 974	
Demographic variables							
Moderate anemia (%)	4.2	7.9	4.5	1.9	3.5	4.1	<0.0001
Age (years)	54.1 (14.76)	52.3 (15.49)	48.1 (15.10)	49.1 (15.11)	55.9 (16.05)	52.3 (15.85)	<0.0001
History of diabetes (%)	18.9	19.6	18.9	10.5	14.2	15.8	<0.0001
Laboratory parameters							
Serum phosphorus (mg/dL)							
Mean (SD)	3.5 (0.63)	3.4 (0.63)	3.5 (0.64)	3.5 (0.61)	3.5 (0.63)	3.5 (0.63)	<0.0001
Median (IQR)	3.5 (3.1, 3.9)	3.4 (3.0, 3.8)	3.5 (3.1, 3.9)	3.5 (3.1, 3.9)	3.5 (3.1, 3.9)	3.5 (3.0, 3.9)	
Hemoglobin (g/dL)							
Mean (SD)	13.6 (1.54)	13.0 (1.56)	13.7 (1.58)	14.1 (1.46)	13.8 (1.51)	13.7 (1.56)	<0.0001
Median (IQR)	13.7 (12.7, 14.7)	13 (12.1, 14.0)	13.7 (12.7, 14.8)	14.1 (13.1, 15.1)	13.8 (12.9, 14.8)	13.7 (12.7, 14.8)	
CKD-EPI eGFR (mL/min/1.73 m^2)							
Mean (SD)	86.0 (23.67)	77.5 (23.28)	93.1 (23.17)	87.0 (22.21)	80.8 (22.36)	84.8 (23.43)	<0.0001
Median (IQR)	89.2 (70.3, 103.3)	77.3 (60.5, 94.2)	96.5 (79.0, 109.9)	88.4 (72.1, 103.3)	81.8 (65.2, 97.1)	86.2 (68.6, 102.5)	
Serum albumin (g/dL)							
<4.0	51%	67.8%	62.8%	41.5%	55.1%	56.1%	<0.0001
≥4.0	49%	32.2%	37.2%	58.5%	44.9%	43.9%	
Ferritin (ng/mL)							
<100	39.3%	48.8%	56.5%	50.6%	51.2%	51.2%	<0.0001
≥100	60.7%	51.2%	43.5%	49.4%	48.8%	48.8%	
Iron saturation (%)							
<20	31.7	41.1	40	33.3	37.6	37.8	<0.0001
≥20	68.3	58.9	60	66.7	62.4	62.2	

Mean and median values of phosphorus, hemoglobin and eGFR are shown. Blacks had the lowest mean phosphorus levels with lower hemoglobin levels and the greatest rates of moderate anemia. Values are mean (SD) when appropriate. Categorical variables were analyzed using χ^2 or Fisher's exact test and continuous variables using independent samples t-test, Kruskal–Wallis or Mann–Whitney U-test, as appropriate.
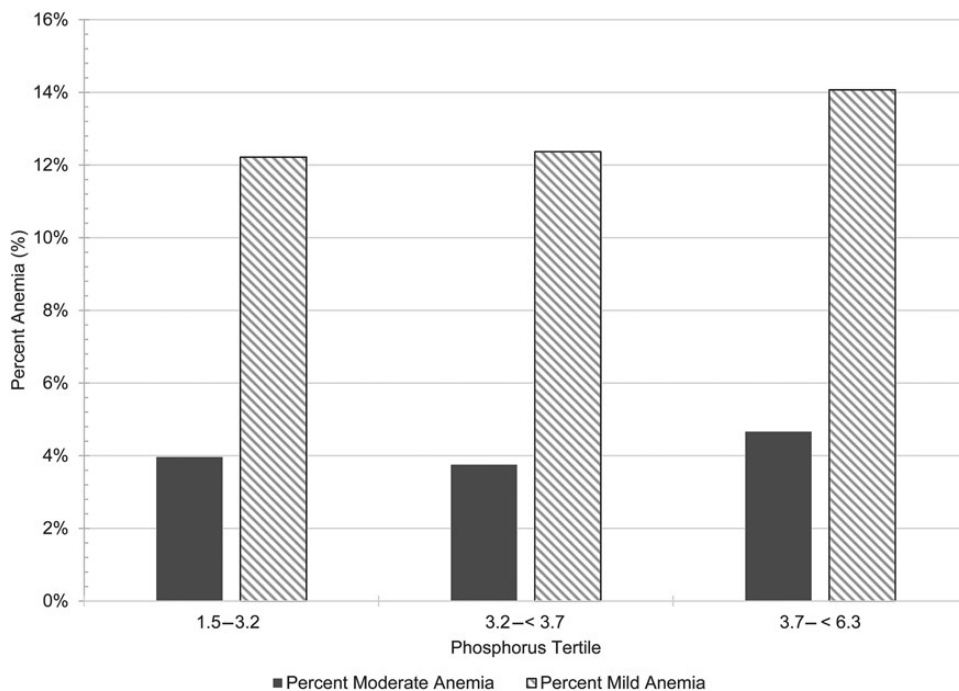
FIGURE 2: Percentage of anemia across phosphorus tertiles. Phosphorus tertiles were categorized as: first (1.5 to <3.2 mg/dL), second (3.2 to <3.7 mg/dL) and third (3.7 to <6.3 mg/dL). The highest percentages of mild and moderate anemia were noted in the third tertile.

DISCUSSION

Higher serum phosphorus levels within the upper limits of normal have been associated with morbidities as well as adverse outcomes, including cardiovascular disease, progression to end stage renal disease (ESRD) and mortality even among those with normal kidney function [21–31]. Hyperphosphatemia and its association with anemia have been described

Table 3. Multivariable adjusted OR for moderate anemia

	Unadjusted			Demographics, eGFR- adjusted			Fully adjusted[a]		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Phosphorus (mg/dL)[b]									
1.5 to <3.2	1.06	1.00–1.13	<0.01	—	—	—	1.01	0.84–1.21	0.93
3.2 to <3.7	1.00	Reference	—	—	—	—	1.00	Reference	—
3.7 to <6.3	1.26	1.18–1.33	<0.01	—	—	—	1.26	1.07–1.48	<0.01
Every 0.5 mg/dL increase	1.14	1.09–1.18	<0.01	1.09	1.05–1.13	<0.01	1.16	1.04–1.29	<0.01
Age (years)									
18–49	1.00	Reference	—	1.00	Reference	—	1.00	Reference	—
50–59	0.79	0.74–0.84	<0.01	0.65	0.60–0.70	<0.01	0.98	0.80–1.21	0.86
60–69	0.87	0.80–0.93	<0.01	0.62	0.57–0.68	<0.01	1.01	0.81–1.27	0.91
≥70	1.49	1.40–1.59	<0.01	0.98	0.90–1.06	<0.01	1.15	0.92–1.45	0.22
Sex									
Male	1.00	Reference	—	1.00	Reference		1.00	Reference	—
Female	2.39	2.25–2.54	<0.01	2.37	2.23–2.52	<0.01	1.76	1.50–2.06	<0.01
Race									
White	1.00	Reference	—	1.00	Reference	—	1.00	Reference	—
Black	2.37	2.22–2.53	<0.01	2.14	2.00–2.29	<0.01	1.13	0.94–1.36	0.19
Hispanic	1.31	1.23–1.40	<0.01	1.36	1.27–1.46	<0.01	1.11	0.91–1.34	0.31
Asian/PI[c]	1.23	1.11–1.37	<0.01	1.26	1.13–1.40	<0.01	1.53	1.15–2.01	<0.01
Other	0.54	0.49–0.60	<0.01	0.62	0.56–0.69	<0.01	0.53	0.40–0.70	<0.01
Diabetes									
No	1.00	Reference	—	1.00	Reference		1.00	Reference	—
Yes	2.00	1.89–2.12	<0.01	1.87	1.76–1.98	<0.01	1.17	1.00–1.37	0.05
Ferritin (ng/ mL)									
<100	1.46	1.35–1.58	<0.01	—	—	—	0.97	0.83–1.13	0.66
≥100	1.00	Reference	—	—	—	—	1.00	Reference	—
Iron saturation (%)									
<20	3.52	3.26–3.80	<0.01	—	—	—	2.57	2.23–2.98	<0.01
≥20	1.00	Reference	—	—	—	—	1.00	Reference	—
Albumin (g/dL)									
<4.0	5.18	4.57–5.88	<0.01	—	—	—	3.21	2.67–3.87	<0.01
≥4.0	1.00	Reference	—	—	—	—	1.00	Reference	—
eGFR[d] (×10 U)	0.92	0.91–0.93	<0.01	0.92	0.90–0.93	<0.01	0.94	0.91–0.97	<0.01

In the fully adjusted model, OR was adjusted for age, sex, race, history of diabetes, ferritin, iron saturation, albumin and renal function. OR was the highest in the third phosphorus tertile and significant in females, Asians, diabetics and individuals with low iron saturation and low albumin.

[a]In the fully adjusted model, ORs have been adjusted for demographics, eGFR, serum albumin, ferritin and iron saturation.
[b]Phosphorus tertiles were modeled separately; other covariate estimates are reported for the model with continuous phosphorus.
[c]Asian/PI, Asian/Pacific Islander race.
[d]eGFR (per 10 mL/min/1.73 m^2).

in dialysis and kidney transplant populations [9, 11, 12]. A recent study suggested that individuals with normal kidney function who have higher phosphorus levels but within the normal range were associated with a higher risk of anemia [17].

Main findings

We found an association between serum phosphorus levels and both mild and moderate anemia within a large diverse population without CKD and early CKD. This association remained after adjustments for demographics, renal function, serum albumin and iron status. A dose-dependent effect was seen across ranges of serum phosphorus, with both risk of anemia and absolute hemoglobin levels. This was demonstrated by the correlation plots of phosphorus and hemoglobin as well as by linear regression modeling for anemia risk. Every 0.5 mg/dL phosphorus level increase demonstrated a 16% greater likelihood for moderate anemia. Although serum phosphorus levels >3.5 mg/dL were associated with a greater likelihood for moderate anemia, levels <2.0 mg/dL also showed a trend toward anemia, suggesting a

curvilinear relationship across ranges of serum phosphorus. Every 10 U increase in eGFR was associated with a 6% decrease in moderate anemia risk.

Additional risk factors for anemia included female sex, Asian race, diabetes, low albumin and low iron saturation. We found a greater likelihood for anemia in all females compared with males, but highest in females aged 18–49 and 70 years and older. Our study also showed that anemia risk increases with low phosphorus levels, low albumin and older age (70+ years in females and 50+ years in males), which may be explained by malnutrition and poor nutrient intake. Overall, our findings were drawn from one of the largest and ethnically diverse populations to date evaluating the relationship between phosphorus and anemia among a non-dialysis population within a real world clinical environment.

Mechanism

Several mechanisms for hyperphosphatemia and anemia have been proposed for CKD and ESRD populations. Erythropoietin
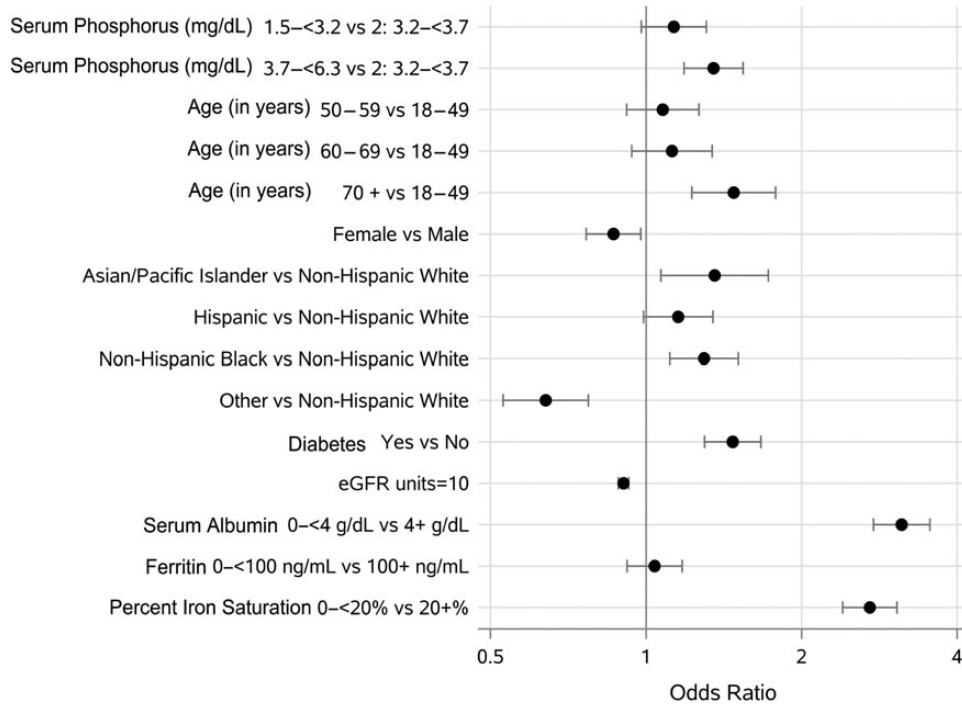
FIGURE 3: Forest plot of fully adjusted OR for moderate anemia. OR was adjusted for demographics, eGFR, serum albumin, ferritin and iron saturation. A significant likelihood of moderate anemia was detected in the third phosphorus tertile, females, Asians, diabetics and individuals with low albumin and low iron saturation. Other race and every 10 U increase in eGFR were associated with decreased anemia.
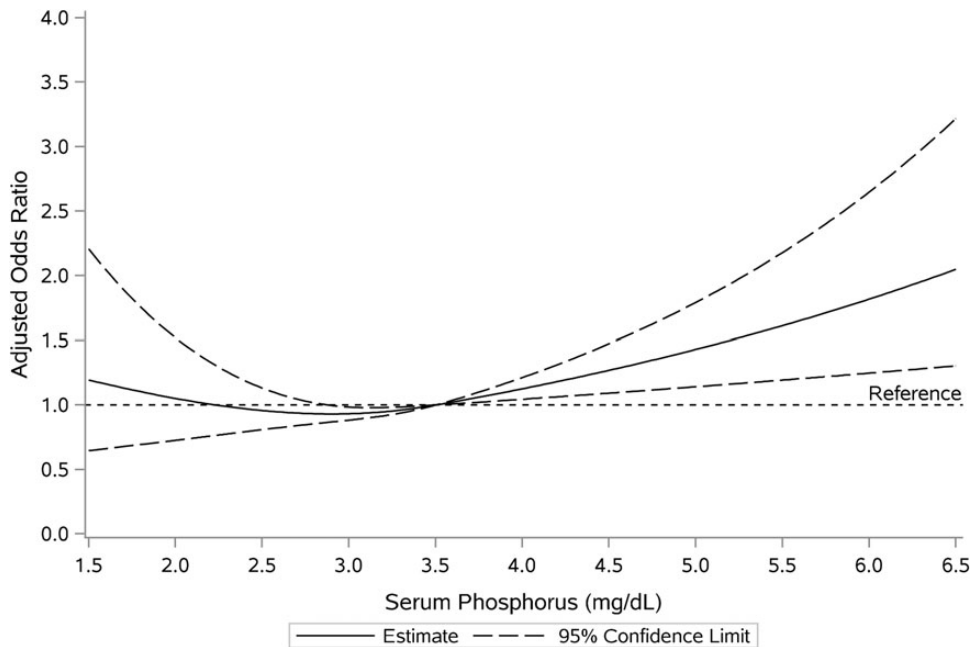
FIGURE 4: Cubic spline plot of serum phosphorus and moderate anemia likelihood. OR was adjusted for age, sex, race, history of diabetes, renal function, serum albumin, ferritin and iron saturation. There was a significant likelihood of moderate anemia with phosphorus levels >3.5 mg/dL and a trend toward higher anemia likelihood with phosphorus levels <2.0 mg/dL.

deficiency, inflammation and oxidative stress have been implicated as potential factors linking hyperphosphatemia and anemia [7]. Kovesdy *et al.* [7] suggested that high serum phosphorus may lead to higher polyamines production, which can function as uremic toxins inhibiting erythropoiesis. In the presence of high serum phosphorus, vascular calcification can form including within the renal arteries, which may eventually result in erythropoietin deficiency and anemia [3, 10]. Hyperphosphatemia in CKD can also cause a decrease in vitamin D synthesis, resulting in hypocalcemia and elevated PTH [2].

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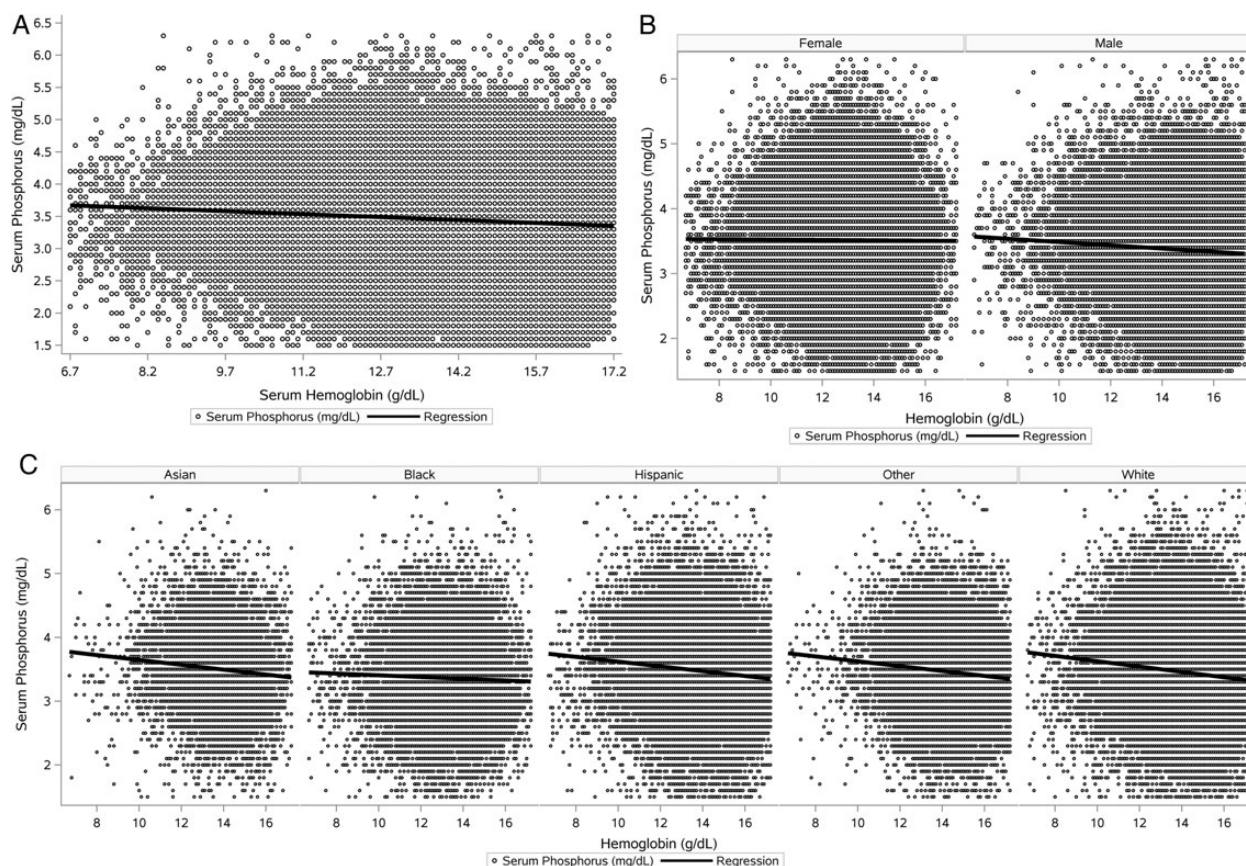
A) There is a negative correlation (p value < 0.0001) between hemoglobin and phosphorus within the cohort.
B) There is a negative correlation between hemoglobin and phosphorus for males but not for females (p value < 0.0001).
C) There is a negative correlation between hemoglobin and phosphorus for all ethnic races (p value <0.0001).

FIGURE 5: Correlation analyses between hemoglobin and phosphorus. There was a significant negative correlation detected between phosphorus (mg/dL) and hemoglobin (g/dL) for (**A**) the entire cohort population, (**B**) males but not females and (**C**) all ethnic race groups including Asians, blacks, Hispanics, whites and other.

Elevated PTH, in turn, has been shown to directly inhibit erythropoiesis, induce hemolysis and cause bone marrow fibrosis in CKD [8–12]. The mechanisms underlying serum phosphorus and anemia in those with early CKD and normal kidney function are less certain.

FGF-23 may play a role in the mechanism underlying the association between hyperphosphatemia and anemia. High serum phosphorus itself has been shown to increase FGF-23 levels, which in turn is associated with suppression of klotho [32, 33]. In murine models, low klotho levels are associated with poor outcomes including early senescence, cell toxicity, premature aging and vascular calcifications [34]. Low klotho also causes a deficiency in activated vitamin D [15]. Vitamin D deficiency in itself has been implicated with anemia [35, 36].

Limitations and strengths

Our study has several potential limitations. The information available and presented in this study was drawn from experiences of a real-world clinical practice environment. Thus, there is a bias toward those individuals who had indications for concurrent hemoglobin, creatinine and phosphorus values to be drawn. This cohort may represent a sicker population with unhealthy lifestyles who sought medical care. In addition,

certain information that would provide insight and contribute to confounding was not available for all individuals. For instance, experimental laboratory measurements such as FGF-23, klotho, hepcidin or erythropoietin levels were never measured for our study cohort. We had data on a very small portion of the study cohort with regard to serum PTH, vitamin D, calcium and other components of bone mineral disorders. Elevated PTH levels, in particular, may have contributed to residual confounding, especially because PTH has been associated with erythropoiesis in CKD [8–12]. We also determined *a priori* that the first phosphorus value available for each individual would be used for our cross-sectional analysis. Phosphorus measurements were drawn and performed throughout different working times of the day in which circadian variations of phosphorus occur. Serum phosphorus values are lowest in the morning and higher later in the day [37]. Given the large size of our study cohort, the variations in phosphorus measurements are likely minimized.

Our study lacked information on medication use among the study cohort. The use of medications such as phosphorus binders, erythropoietin, steroids or iron salts was not ascertained. Among CKD patients, certain phosphorus binders have been associated with improved anemia parameters [38–40]. Nutritional

supplements and dietary phosphorus intake were also not available to evaluate their effects on serum phosphorus levels. We could not definitively exclude renal function as a causative mechanism for anemia and hyperphosphatemia as we included those with eGFR > 30 mL/min/1.73 m^2, though we did attempt to adjust for renal function in our multivariable regression models. Finally, correlation does not indicate causation, and the results of our cross-sectional analyses should not be interpreted as causal association between phosphorus and anemia.

One strength of our study is that it is one of the largest non-dialysis populations to date ($N = 155\,974$), evaluating the association between phosphorus and anemia. Our cohort was a racially and ethnically diverse, nationally representative population observed within a real world clinical environment. Compared with previous studies, we had comprehensive information on comorbidities and the laboratory, pharmacy and health utilization of our study population. This enabled us to adjust for potential confounders and to exclude many secondary causes of anemia.

Implications/conclusion

Higher phosphorus levels (>3.5 mg/dL) were associated with a greater likelihood for moderate anemia in individuals with early CKD stages and those with relatively intact kidney function. We also observed a trend for lower phosphorus levels (<2.0 mg/dL). Our study suggests that phosphorus may be a biomarker associated with anemia and/or play a role in hematopoiesis. Understanding the impact of phosphorus on hemoglobin levels may have implications on the prevention of anemia in individuals with non-CKD or early stage CKD. Theoretically, a 'treatment' effect of moving individuals with phosphorus levels of 4.0 mg/dL or higher to levels of 3.5 mg/dL or lower may lead to possible prevention of moderate anemia. Given our findings, 87.1 (95% CI 70.7–112.8) individuals would need to have this 0.5 mg/dL lowering of phosphorus to prevent one excess case of moderate anemia [34]. Within our population, a 0.5 mg/dL lower shift in the distribution of phosphorus could have correlated with prevention of anemia in hundreds of individuals. Anemia has already been associated with adverse outcomes, including higher incidences of coronary events, stroke, progression to ESRD and death. Our study cohort established using an electronic health-record-based approach has the potential to describe many clinical outcomes related to CKD-bone mineral disorders across different ranges of renal function.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxford journals.org.

AUTHORS' CONTRIBUTIONS

J.J.S. and M.B. had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis ; study concept and design: J.J.S., L.T. and M.B.; acquisition, analysis or interpretation of data: J.J.S., L.T. and M.B.; drafting of the manuscript: L.T. and J.J.S.; critical revision of the manuscript for important intellectual content: C.M.R., E. S., K.K.-Z. and S.J.J.; statistical analysis: M.B.; administrative, technical or material support: S.J.J. and study supervision: J.J.S. and S.J.J.

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