Association between trace element concentrations and anemia in patients with chronic kidney disease: a cross-sectional population-based study

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ABSTRACT

Anemia is common in chronic kidney disease (CKD) and may be affected by trace element concentrations. While the concentrations of trace elements are known to be altered in CKD, the relationship between trace element and hemoglobin concentrations has not been systematically investigated in a large cohort. This study aims to examine associations between trace element concentrations and anemia in patients with CKD. Data from the National Health and Nutrition Examination Survey collected from 2011 to 2014 were used for this analysis. The participants who were more than 20 years old were included. A total of 3057 participants were included; the final cohort was divided into two groups based on CKD status. The concentrations of hemoglobin, iron, zinc, and manganese were significantly lower in participants with than without CKD (all $p<0.05$). Multivariate analyses showed that in patients without CKD, hemoglobin concentrations correlated positively with iron, zinc, and cadmium ($\beta=0.005$, 0.009, and 0.33, respectively), but correlated negatively with copper levels ($\beta=-0.002$). In patients with CKD, hemoglobin concentrations correlated positively with cadmium and selenium, but negatively with copper levels ($\beta=0.57$, 0.007, and −0.008, respectively). The serum iron concentration was found to correlate positively with zinc, cadmium, and selenium, but negatively with copper and manganese concentrations in the total study population (all $p<0.05$). The associations between serum concentrations of trace elements and hemoglobin differ between patients with and without CKD. Further investigations are warranted to determine whether patients with CKD have distinct trace element requirements.

Significance of this study

What is already known about this subject?
- Anemia is common among patients with chronic kidney disease (CKD).
- Anemia is associated with increased morbidity and mortality in patients with CKD.
- Trace element concentrations are altered in patients with CKD.

What are the new findings?
- Hemoglobin concentration correlates positively with iron, zinc, and cadmium levels in patients without CKD but with cadmium and selenium levels in patients with CKD.
- Hemoglobin concentration correlates negatively with copper levels in patients with and without CKD.
- In the total population, serum iron concentration correlates positively with zinc, cadmium, and selenium, but negatively with copper and manganese concentrations.

How might these results change the focus of research or clinical practice?
- These findings indicate that further investigations are warranted to determine whether patients with CKD have unique trace element requirements.

INTRODUCTION

Anemia, characterized by abnormally low blood hemoglobin concentration, is common among patients with chronic kidney disease (CKD).[1 2] Most patients beginning dialysis have a hemoglobin concentration below the recommended range.[3] Importantly, anemia is associated with increased morbidity and mortality in patients with CKD, regardless of the disease stage.[4 5] Patients with a hematocrit <33% are at increased risk of hospitalization and death, and a hematocrit in the range of 33% to 36% appears to be optimal for reducing morbidity and mortality in patients with CKD.[6] Thus, maintaining optimal hemoglobin levels is clearly important to the health of patients with CKD.

Red blood cell proliferation is regulated by the hormone erythropoietin, which is released from the kidneys in response to low tissue concentrations of oxygen.[7] Failing kidneys produce less erythropoietin, causing a decrease in red blood

cell production that contributes to the anemia commonly associated with CKD. Hemoglobin is composed of four globin chains, each of which is attached to an iron-containing heme group. Hemoglobin synthesis requires the availability of iron and the presence of heme. In turn, heme production involves the activity of an enzyme that is zinc dependent.[7] Thus, factors that affect the availability of iron and zinc may also affect the synthesis of heme and hemoglobin. Copper, zinc, cobalt, manganese, and cadmium, because of their similarity in physiochemical characteristics to iron, can interfere with normal iron metabolism.[7]

Trace element concentrations are known to be altered in patients with CKD,[8 9] resulting from a variety of factors. Dietary restrictions and anorexia lead to insufficient micronutrient intake, while diuretics and renal replacement therapy lead to their excessive losses, unpredictable absorption, and impaired metabolism.[9] In patients with CKD, zinc deficiency is prevalent and associated with refractory anemia.[10] Evidence also suggests that copper deficiency is associated with anemia and the presence of erythropoietin hyporesponsiveness in these patients.[11] Studies of cadmium and manganese in CKD are rare; however, several studies suggest an association between these trace elements and CKD. High blood cadmium levels are associated with malnutrition and higher mortality in patients on maintenance hemodialysis.[9 12] Predialysis patients with chronic renal failure are reported to have elevated levels of circulating manganese,[13] while another study reports that blood manganese concentration is positively associated with hemoglobin level in patients with CKD.[14]

While these studies suggest the importance of trace elements in CKD-associated anemia, comparison between them is limited by differences in experimental conditions. Trace element concentrations were estimated from different sources (blood, urine, or tissue), between which the normal levels of trace elements vary. In addition, the sample sizes of many of these studies were low, with few having cohorts over 100. Given the complex interactions between these trace elements in the metabolic events leading to anemia, the investigation of multiple trace elements in one study is needed. Conclusive data regarding the correlations between trace elements and anemia in patients with CKD could be used to better address the specific needs of this population of patients.

The purpose of this large-cohort, population-based study is to investigate the associations between trace elements and anemia in patients with CKD using medical records in a large national database. The results may assist physicians in evaluating and treating anemia in patients with CKD.

MATERIALS AND METHODS

Data source

Data from the National Health and Nutrition Examination Survey (NHANES) collected from 2011 to 2012 and 2013–2014 cycles were used for this analysis. The NHANES program began in the USA in the early 1960s, and has been conducted as a series of surveys focusing on different population groups and health topics. Samples for NHANES surveys are selected to represent the US population of all ages. Further information about background, design, and operation are available on the NHANES website (http://wwwn.cdc.gov/nchs/nhanes). All of the NHANES data are de-identified, and analysis of the data does not require Institutional Review Board approval or informed consent by subjects.

Study population

The present study population consisted of NHANES participants over 20 years of age who had data regarding the estimated glomerular filtration rate (eGFR) within the selected time period. CKD was defined as eGFR <60 as calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.[15 16] The mean eGFR was 98.33 in the non-CKD group and 46.68 in the CKD group.

Participants who were pregnant, had a history of any malignancy, or had missing data regarding hemoglobin or eGFR were excluded from the analysis. The distribution of baseline characteristics between included and excluded participants is shown in online supplementary table 1.

Study variables

Laboratory data were examined for associations with hemoglobin concentration included trace essential elements (iron, zinc, copper, cadmium, selenium, and manganese), CKD stage, red blood cell, indices of mean cell volume (MCV), red cell distribution width (RDW), and glycohemoglobin. Demographic and clinical data analyzed included age, sex, race/ethnicity, body mass index (BMI), self-reported health conditions (congestive heart failure, coronary heart disease/myocardial infarction, and cigarette smoking).

Demographic data

The Family and Sample Person Demographics questionnaires were collected in the participants' homes by trained interviewers using the Computer-Assisted Personal Interviewing system.

Age, sex, and race/ethnicity were recorded using interviewer-administered questionnaires. Race/ethnicity was self-reported as Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other Race—including multiracial.

Laboratory data

Blood and urine specimens were collected at NHANES Mobile Examination Centers (MECs). Whole blood and urine specimens were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, and Centers for Disease Control and Prevention for analysis. Complete descriptions of the collection and analytical methods are available in the Laboratory data section of the NHANES database (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/BIOPRO_G.htm and https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/PBCD_G.htmref).

Body mass index

BMI data were recorded using the "Body Measures" recommendations in the NHANES Examination Protocol. The body measurement data were collected in an MEC by trained health technicians. Details of the examination are available on the NHANES website (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/BMX_G.htm). For this study, participants were categorized based on WHO criteria: underweight (BMI<18.5 kg/m^2), normal

(BMI=18.5–24.9 kg/m^2), overweight (BMI=25–29.9 kg/m^2), and obese (BMI≥30.0 kg/m^2).

Health conditions

Medical history was self-reported and collected by interviewer-administered questionnaires. Details of the questionnaire are available on the NHANES website (http://wwwn.cdc.gov/Nchs/Nhanes/2007-2008/MCQ_E.htm). Relevant conditions for the current study were congestive heart failure and coronary heart disease/myocardial infarction. These conditions were selected because the reported cardiovascular morbidity and mortality are high in patients with CKD, with particular contributions from coronary artery disease, myocardial infarction, and congestive heart failure.[17]

Smoking status was recorded using interviewer-administered questionnaires (Smoking—Cigarettes Use). Participants were categorized as a current regular smoker and never regular smoker. Current regular smoker was defined as a participant who smoked at least 100 cigarettes in life and still smokes regularly at the time of answering questionnaires. Never regular smoker included non-smoker and participant who never smoked cigarettes regularly.

Statistical analysis

The following equation was used to estimate sample size:

$$n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2}$$

Where z is the z score, ε is the margin of error, N is population size, and p̂ is the population proportion. We assumed Z=1.96; ε=0.05; p̂=0.3.

Therefore, n=322.69 based on the above equation is a sufficient number of subjects to include in the analysis. A total of 3057 subjects is equivalent to a population-based sample size of 193 660 664 persons, as explained in the NHANES documentation.

For the analysis, participants were grouped according to kidney function stage, with stages 1 or 2 placed in the non-CKD group and those with stages 3, 4, or 5 placed in the CKD group. The NHANES uses a complex survey design to assure national representation.[18] Weighting variables including pseudo-stratum (SDMVSTRA), pseudo-cluster (SDMVPSU), and subsample-A-weight (WTSA2YR) were used in all analyses. WTSA2YR was selected as the sample weight because data on serum zinc, serum copper, and serum selenium were measured in a one-third subsample of persons 6 years and older in this study. Continuous variables are reported as the mean and SE by the SAS Survey Means procedure. For categorical variables, the SAS SurveyFreq was used to calculate a number and weighted proportion of persons in the USA. Frequency distributions between categorical variables were assessed using the χ^2 test. Continuous variables were testing using ANOVA. All tests were applied with discharge weights to account for the NHANES sampling method. The relationships between variables and hemoglobin concentration were examined using survey-weighted linear regression. Pearson correlation analysis was used to determine the correlation between serum iron and other trace elements. Significant variables revealed by univariate analysis were subsequently analyzed by multivariate analysis. A p value <0.05 was considered to indicate statistical significance. All analyses were performed using SAS software V.9.4 (SAS Institute, Cary, North Carolina, USA).

Table 1 Characteristics of the study population (n=3057, weighted n=193 660 664)

	Non-CKD	CKD	
	n=2853	n=204	P value
Demographics			
Age ≥50 years	1157 (38.29)	193 (92.69)	**<0.0001**
Sex, female	1432 (50.43)	104 (62.61)	**0.02**
Race			**<0.0001**
Mexican American	364 (9.43)	11 (2.78)	
Other Hispanic	283 (6.69)	19 (5.08)	
Non-Hispanic white	1083 (64.16)	111 (78.88)	
Non-Hispanic black	637 (11.41)	44 (8.29)	
Other races	486 (8.31)	19 (4.96)	
Body measures			
BMI*			0.32
Normal	842 (29.44)	53 (23.66)	
Underweight	47 (1.38)	2 (0.90)	
Overweight	893 (33.59)	63 (32.27)	
Obese	1037 (34.67)	83 (42.03)	
Unknown/missing	34 (0.93)	3 (1.14)	
Laboratory data			
MCV (fL), mean±SE	89.71±0.14	90.46±0.34	**0.03**
RDW (%), mean±SE	13.19±0.04	13.76±0.11	**<0.0001**
Glycohemoglobin (%), mean±SE	5.57±0.02	6.11±0.09	**<0.0001**
Health conditions			
Congestive heart failure			**<0.0001**
No	2802 (98.44)	171 (85.13)	
Yes	46 (1.48)	31 (14.08)	
Unknown/missing			
Coronary heart disease/myocardial infarction	117 (4.34)	52 (23.36)	**<0.0001**
Cigarette smoking	536 (20.26)	41 (18.89)	0.68

Continuous data are presented as mean±SE; categorical data are presented as unweighted counts (weighted proportion). Numbers in bold indicate significant difference between groups (p<0.05).
*BMI <18.5 kg/m^2 was defined as underweight, BMI between 18.5 and 24.9 kg/m^2 as normal, BMI between 25 and 29.9 kg/m^2 as overweight, and BMI ≥30.0 kg/m^2 as obese.
BMI, body mass index; CKD, chronic kidney disease; MCV, mean cell volume; RDW, red cell distribution width.

RESULTS

Participant characteristics

Review of the NHANES database from 2011 and 2014 identified 10,907 participants who were over 20 years of age and had complete laboratory data. After excluding participants with kidney dialysis or missing data regarding eGFR, 9196 persons remained. The laboratory examinations required for data analysis had been performed for only one-third of these patients. Hence, after exclusions for missing laboratory data, 3057 participants remained in the final cohort. A total of 3057 subjects is equivalent to a population-based sample size of 193,660,664.

Participant demographic and clinical characteristics are summarized in tables 1 and 2. Of the 3057 eligible participants, 51.09% were women, more than half were under 50 years old (58.73%) and non-Hispanic white (64.97%),

Table 2 Hemoglobin concentration and trace element levels

	Non-CKD	CKD	
	n=2853	n=204	P value
Hemoglobin (g/dL)	14.22±0.05	13.46±0.14	**<0.0001**
Trace elements			
Serum iron (µg/dL)	87.77±0.95	78.02±2.72	**0.002**
Serum zinc (µg/dL)	82.78±0.67	76.86±1.29	**<0.0001**
Serum copper, total (µg/dL)	117.71±1.22	112.12±3.12	0.16
Blood cadmium (µg/L)	0.45±0.01	0.54±0.05	0.11
Serum selenium (µg/L)	130.68±0.83	129.53±2.13	0.53
Blood manganese (µg/L)	9.69±0.08	8.83±0.16	**<0.0001**

All data are presented as mean±SE. Numbers in bold indicate significant difference between groups (p<0.05).
CKD, chronic kidney disease.

slightly less than half were obese (35.08%), and most of the participants (93.32%) did not have CKD. The mean eGFR was 98.33 in the non-CKD group and 46.68 in the CKD group.

Significant differences were observed in age, sex, race, MCV, RDW, glycohemoglobin, congestive heart failure, and coronary heart disease/myocardial infarction between participants without CKD and without CKD (all p≤0.03).

The mean hemoglobin concentration was significantly higher in patients without CKD than in those with CKD (14.22±0.05 g/dL vs 13.46±0.14 g/dL, p<0.0001). The mean concentrations of serum iron, serum zinc, and manganese were also significantly higher in patients without CKD than in those with CKD (iron: 87.77±0.95 g/dL vs 78.02±2.72 g/dL, p=0.002; serum zinc: 82.78±0.67 g/dL vs 76.86±1.29 g/dL, p<0.0001; blood manganese: 9.69±0.08 g/dL vs 8.83±0.16 g/dL, p<0.0001) (table 2).

Associations between trace element and hemoglobin concentrations

Univariate and multivariate analyses of the associations between trace elements and hemoglobin concentration are shown in table 3. Univariate analysis showed that hemoglobin concentration was significantly associated with serum iron, zinc, copper, cadmium, selenium, and manganese levels among participants without CKD (β=0.01, 0.02, –0.02, 0.14, 0.01, and −0.06, respectively). In patients with CKD, hemoglobin concentration was significantly associated with serum copper, cadmium, and selenium levels (β=−0.02, 0.41, and 0.02, respectively).

Multivariate analysis showed that serum iron, zinc, and cadmium levels positively correlated with hemoglobin concentration in patients without CKD (β=0.005, 0.009, and 0.33, respectively) after adjustment for sex, race, BMI, MCV, RDW, congestive heart failure, coronary heart disease/myocardial infarction, and cigarette smoking. In patients without CKD, serum cadmium and selenium levels correlated positively with hemoglobin concentration (β=0.57 and 0.007, respectively) after adjustment for sex, race, BMI, MCV, RDW, and congestive heart failure (table 3).

Hemoglobin concentrations according to trace element concentrations

The relationship between hemoglobin and trace element concentrations is shown in table 4. Patients with above normal levels of iron, zinc, and selenium had significantly higher concentrations of hemoglobin (p<0.0001). Patients with below normal levels of copper and manganese had significantly higher concentrations of hemoglobin (p<0.0001). The relationship between serum iron and other trace element concentrations in the total study population is shown in online supplementary table 2. Serum iron concentration was found to correlate positively with zinc, cadmium, and selenium, but negatively with copper and manganese concentrations (all p<0.05).

DISCUSSION

This study uses a population-based database to examine the associations between the concentrations of hemoglobin and trace elements in 3057 participants with or without CKD. In our study cohort, the concentrations of hemoglobin, iron, zinc, and manganese were significantly lower in participants with CKD than in those without. We observed that hemoglobin concentration correlates positively with iron, zinc, and cadmium levels in patients without CKD, and with cadmium and selenium levels in patients with CKD. We found that hemoglobin concentration correlates negatively with copper levels in patients with and without CKD. Examination of potential influences between trace elements on their concentrations in the total study population

Table 3 Univariate and multivariate analysis of the association between hemoglobin and trace element concentrations

	Non-CKD		CKD	
	Univariate	Multivariate*	Univariate	Multivariate†
	β±SE	β±SE	β±SE	β±SE
Trace elements				
Serum iron (µg/dL)	**0.01±0.001**	**0.005±0.94**	0.01±0.001	–
Serum zinc (µg/dL)	**0.02±0.002**	**0.009±0.002**	0.02±0.01	–
Serum copper, total (µg/dL)	**–0.02±0.001**	**–0.002±0.001**	**–0.02±0.001**	**–0.008±0.003**
Blood cadmium (µg/L)	**0.14±0.05**	**0.33±0.05**	**0.41±0.18**	**0.57±0.21**
Serum selenium (µg/L)	**0.01±0.001**	0.002±0.001	**0.02±0.001**	**0.007±0.003**
Blood manganese (µg/L)	**–0.06±0.01**	0.02±0.01	0.05±0.04	–

Numbers in bold indicate significant difference (p<0.05).
*Model was adjusted for sex, race, BMI, MCV, RDW, congestive heart failure, coronary heart disease/myocardial infarction, and cigarette smoking.
†Model was adjusted for sex, race, BMI, MCV, RDW, and congestive heart failure.
β, coefficient; BMI, body mass index; CKD, chronic kidney disease; MCV, mean cell volume; RDW, red cell distribution width.

Table 4 Hemoglobin concentrations according to trace element ranges

	Hemoglobin concentration (g/dL)			
	Trace element below normal	Trace element normal	Trace element above normal	
	Mean±SE	Mean±SE	Mean±SE	P value*
Serum iron (µg/dL)†	13.28±0.08	14.41±0.05	14.90±0.21	**<0.0001**
Serum zinc (µg/dL)‡	13.52±0.10	14.25±0.05	14.72±0.13	**<0.0001**
Serum copper, total (µg/dL)§	14.97±0.25	14.36±0.05	13.32±0.07	**<0.0001**
Blood cadmium (µg/L)¶	14.14±0.05	14.25±0.12	NA	0.37
Serum selenium (µg/L)**	NA	14.11±0.05	14.62±0.09	**<0.0001**
Blood manganese (µg/L)††	13.67±0.27	14.23±0.05	12.81±0.19	**<0.0001**

Trace element concentration ranges used to stratify the hemoglobin data:
*Numbers in bold indicate significant difference between groups (p<0.05).
†Below normal, <60 µg/dL; normal, 60–170 µg/dL; above normal, >170 µg/dL.[26]
‡Below normal, <66 µg/dL; normal, 66–110 µg/dL; above normal, >110 µg/dL.[27]
§Below normal, <70 µg/dL; normal, 70–140 µg/dL; above normal, >140 µg/dL.[28]
¶Below normal, <0.5 µg/L; normal, 0.5–2 µg/L; above normal, >2 µg/L.[29]
**Below normal, <70 µg/L; normal, 70–150 µg/L; above normal, >150 µg/L.[30]
††Below normal, <4.7 µg/L; normal, 4.7–18.3 µg/L; above normal, >18.3 µg/L.[31]
NA, no patients in the group.

revealed that serum iron concentration correlates positively with zinc, cadmium, and selenium but negatively with copper and manganese concentrations.

Of the trace elements examined in this study, zinc is likely the most studied. Studies have shown that hemodialysis patients have low serum zinc concentrations and suggest that zinc supplementation can improve anemia in patients with CKD.[10] We also observed lower serum zinc concentrations in patients with CKD than without CKD, consistent with a previous study showing that the levels of serum zinc had a decreasing trend in the advanced stages of CKD. However, our multivariate analysis revealed a significant association between hemoglobin and zinc concentrations only in patients without CKD. Nevertheless, in an interventional study, Fukushima[19] treated maintenance hemodialysis patients with zinc levels <80 mg/dL (lower than the normal range) with adjuvant zinc therapy and found that zinc treatment was associated with increased hemoglobin concentrations and a reduced dose of erythropoietin. Patients treated with zinc required additional iron supplementation due to the development of iron deficiency. This finding may result from the observed inhibitory effects on intestinal absorption of iron and zinc that these two elements have on each other.[6] Kobayashi *et al*[20] observed no change in hemoglobin concentrations among zinc-treated hemodialysis patients, despite requiring less erythropoiesis-stimulating agent (ESA) on zinc administration. While zinc is clearly important for red blood cell proliferation in patients with CKD, the complexity of its relationship with iron makes the interpretation of study findings difficult to summarize. Further studies are needed to clarify the association between hemoglobin and zinc concentrations in patients with CKD.

As with zinc, we observed that iron levels were higher in patients without than with CKD, and hemoglobin concentration correlated positively with serum iron level in patients without but not with CKD. We hypothesize that the role of iron with respect to hemoglobin concentration may differ to some degree between patients with late-stage CKD and the general healthy population and early-stage patients. Dysregulated iron homeostasis is known to play a central role in the development of anemia in CKD and is a major contributor to resistance to treatment with ESAs,[21] with ESA resistance more common among late-stage patients.[22] The lack of association between iron and hemoglobin in patients with CKD in the present study may be due in part to altered iron metabolism or abnormal intake of trace elements. Additionally, even in the context of normal iron levels, systemic iron deficiency can be present as affected by ferritin levels or transferrin saturation. This phenomenon results from the overproduction of hepcidin in CKD, which inhibits duodenal iron absorption and sequesters iron in macrophages.[20] As we did not have access to ferritin and transferrin data for our cohort, the interpretation of our iron data is limited by this absence.

The blood levels of cadmium did not differ between patients with and without CKD in this study. We observed a positive association between hemoglobin and cadmium concentrations, regardless of CKD status. Cadmium has long been considered renotoxic, with exposure leading to renal failure[23] and subsequent anemia due to the suppression of erythropoietin production. In our study, none of the participants exhibited elevated cadmium levels (table 4), so the negative effects of elevated cadmium on hemoglobin levels were not observed here. In studies that included patients with elevated cadmium levels, associations were noted between cadmium levels and mortality in patients undergoing maintenance dialysis,[9 12] with the authors suggesting that avoidance of environmental exposure to cadmium as much as possible is warranted in chronic peritoneal dialysis patients.

Studies of manganese and selenium with respect to CKD are relatively few. In contrast to our observation that manganese levels did not differ significantly between patients with and without CKD, a recent study observed elevated manganese levels in predialysis patients with CKD as compared with that of healthy controls.[13] The difference in patient cohort profile (predialysis vs dialysis) between our study and theirs may account for these observed differences. Kim *et al*[14] report that blood manganese level is positively associated with hemoglobin concentration in patients with CKD, an association that we did not observe here. However, we did observe a positive correlation between serum iron and serum

concentrations of manganese and selenium in CKD. We observed a positive association between hemoglobin concentration and selenium level, supporting a previous suggestion that selenium supplementation may benefit patients with late-stage CKD, as selenium levels are decreased in patients on long-term dialysis.[24]

The observed negative association between hemoglobin and serum copper concentrations, regardless of CKD status, is consistent with a report that elevated serum copper has a negative effect on hemoglobin production in patients with CKD with and without anemia.[25] These authors suggest that elevated copper affects hemoglobin production by competitively decreasing the absorption of iron. Our observation that iron and copper concentrations are negatively associated is consistent with this hypothesis.

The major strength of this study is the use of a large cohort in the NHANES database. This benchmark national health survey is one of the few population-based surveys that include validated examination measures, biological specimen collection, and measures of health status. Rigorous training in recruitment and data collection ensures high response rates, national representativeness, and high-quality data collection. A major strength of NHANES is the use of a combination of anthropometric measures and biomarkers to assess nutritional status, which decreases bias and errors in measurement. The large multiethnic population cohort allowed for the evaluation of racial and ethnic heterogeneity in the association of trace elements and anemia in patients with CKD. The sample size was large enough for fairly precise prevalence measures at the national level. However, as the survey was conducted in the USA, the results should be validated in cohorts in other countries. Another strength of this study is the use of serum levels of trace elements, which are more accurately determined than urine levels. In addition, no previous study has examined these associations at a population-based level.

This study has several important limitations. Because NHANES is a cross-sectional analysis, no inferences regarding causality can be made. Interview (questionnaire) data are based on self-reports and are therefore subject to potential recall errors, misunderstanding of the question, and a variety of other factors. To overcome these potential biases, we chose to examine primarily objective laboratory data and body measures rather than variables highly subject to individual interpretations or recall. The NHANES database does not provide information regarding intravenous iron infusion, ESA, or recent transfusion history; for this reason, we excluded patients receiving iron supplements. However, patients with CKD included in the analysis may have been receiving some form of iron supplementation for the purpose of medical treatment, and this may have influenced the results to some degree. Lastly, it is possible that patients with non-CKD anemia were included in the analysis.

CONCLUSION

The association between hemoglobin and trace element concentrations differs between patients with and without CKD. These findings warrant further investigation to determine whether patients with CKD have unique trace element requirements.

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REFERENCES

1 Hsu CY. Epidemiology of anemia associated with chronic renal insufficiency. *Curr Opin Nephrol Hypertens* 2002;11:337–41.
2 Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: results from the Third National Health and Nutrition Examination Survey. *J Am Soc Nephrol* 2002;13:504–10.
3 Astor BC, Muntner P, Levin A, *et al*. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988–1994). *Arch Intern Med* 2002;162:1401–8.
4 Ma JZ, Ebben J, Xia H, *et al*. Hematocrit level and associated mortality in hemodialysis patients. *J Am Soc Nephrol* 1999;10:610–9.
5 Xia H, Ebben J, Ma JZ, *et al*. Hematocrit levels and hospitalization risks in hemodialysis patients. *J Am Soc Nephrol* 1999;10:1309–16.
6 Collins AJ, Ma JZ, Ebben J. Impact of hematocrit on morbidity and mortality. *Semin Nephrol* 2000;20:345–9.
7 Garnica AD. Trace metals and hemoglobin metabolism. *Ann Clin Lab Sci* 1981;11:220–8.
8 Chazot C, Jean G, Kopple JD. Can outcomes be improved in dialysis patients by optimizing trace mineral, micronutrient, and antioxidant status? The impact of vitamins and their supplementation. *Semin Dial* 2016;29:39–48.
9 Lee CC, Weng CH, Huang WH, *et al*. Association between blood cadmium levels and mortality in peritoneal dialysis. *Medicine* 2016;95:e3717.
10 Fukushima T, Horike H, Fujiki S, *et al*. Zinc deficiency anemia and effects of zinc therapy in maintenance hemodialysis patients. *Ther Apher Dial* 2009;13:213–9.
11 Higuchi T, Matsukawa Y, Okada K, *et al*. Correction of copper deficiency improves erythropoietin unresponsiveness in hemodialysis patients with anemia. *Intern Med* 2006;45:271–3.
12 Hsu CW, Yen TH, Chen KH, *et al*. Effect of blood cadmium level on mortality in patients undergoing maintenance hemodialysis. *Medicine (Baltimore) 2015;94:e* 1755.
13 Sánchez-González C, López-Chaves C, Gómez-Aracena J, *et al*. Association of plasma manganese levels with chronic renal failure. *Journal of Trace Elements in Medicine and Biology* 2015;31:78–84.
14 Kim M, Koh ES, Chung S, *et al*. Altered metabolism of blood manganese is associated with low levels of hemoglobin in patients with chronic kidney disease. *Nutrients* 2017;9:pii: E1177:1177.
15 National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease. Evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39:S1–266.
16 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–12.
17 Herzog CA, Asinger RW, Berger AK, *et al*. Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2011;80:572–86.
18 Johnson CL, Paulose-Ram R, Ogden CL, *et al*. National health and nutrition examination survey: analytic guidelines, 1999–2010. *Vital Health Stat* 2013;2:1–24.
19 Fukushima T. [The role of zinc in chronic kidney disease]. *Nihon Rinsho* 2016;74:1138–43.

20 Kobayashi H, Abe M, Okada K, *et al*. Oral zinc supplementation reduces the erythropoietin responsiveness index in patients on hemodialysis. *Nutrients* 2015;7:3783–95.
21 Panwar B, Gutiérrez OM. Disorders of iron metabolism and anemia in chronic kidney disease. *Semin Nephrol* 2016;36:252–61.
22 Atkinson MA, Warady BA. Anemia in chronic kidney disease. *Pediatric Nephrology* 2018;33:227–38.
23 Chen J, Du L, Li J, *et al*. Epigallocatechin-3-gallate attenuates cadmium-induced chronic renal injury and fibrosis. *Food and Chemical Toxicology* 2016;96:70–8.
24 Hsieh YY, Shen WS, Lee LY, *et al*. Long-term changes in trace elements in patients undergoing chronic hemodialysis. *Biol Trace Elem Res* 2006;109:115–22.
25 Ganz T, Nemeth E. Iron balance and the role of hepcidin in chronic kidney disease. *Semin Nephrol* 2016;36:87–93.
26 Holm G. Serum iron test. Healthline. https://www.healthline.com/health/serum-iron#normal-results (accessed 3 Oct 2018).
27 Mayo Medical Laboratories. Mayo Clinic. https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/8620 (accessed 3 Oct 2018).
28 Total copper. Health Encyclopedia. University of Rochester, Medical Center. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=total_copper_blood (accessed 3 Oct 2018).
29 Mayo Medical Laboratories. Mayo Clinic. https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8682 (accessed 3 Oct 2018).
30 Mayo Medical Laboratories. Mayo Clinic. https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9765 (accessed 3 Oct 2018).
31 Seladi-Schulman J. Manganese deficiency. Healthline. https://www.healthline.com/health/manganese-deficiency (accessed 3 Oct 2018).