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Research Article

Iron Metabolic Biomarkers and the Mortality Risk in the General Population: A Nationwide Population-Based Cohort Study

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Abstract

Context: Iron is an essential element in the human body and plays a critical role in many physiological and cellular processes. However, the association between iron status and the risk of all-cause or cause-specific mortality has not been well-investigated. And it is unclear whether the association between iron metabolic biomarkers and the risk of mortality differs between people with and without diabetes mellitus (DM).

Objective: This work aimed to investigate associations between iron metabolic biomarkers and all-cause and cause-specific mortality risk in the general population, and heterogeneities in the associations among population with and without DM..

Methods: A total of 29 166 adults from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999 to 2010 were included, with linkage to the National Death Index to December 31, 2019. Cox proportional-hazard models and Fine-Gray subdistribution hazard models were used to estimate associations between iron metabolic biomarkers and outcomes.

Results: During a median follow-up of 18.83 years, 9378 deaths were observed, including 3420 cardiovascular disease (CVD) deaths and 1969 cancer deaths. A significant linear association between serum ferritin (SF) and all-cause mortality was observed among the overall population and those without DM. J-shaped associations between transferrin saturation (TSAT) and all-cause and CVD mortality were observed among all populations. In the overall population, compared to the first quartile (Q1) group, the adjusted hazard ratio (HR) (95% CI) for all-cause mortality was 1.07 (1.00-1.15), 1.05 (0.98-1.12), 1.13 (1.05-1.21) in Q2, Q3, and Q4 groups for SF, while the HR was 0.94 (0.88-0.99), 0.92 (0.86-0.97), and 0.93 (0.88-0.99) for TSAT. In individuals without DM, the adjusted HR of the Q4 of SF were 1.19 (1.03-1.37) for CVD mortality and 1.25 (1.05-1.48) for cancer mortality. In individuals with DM, the adjusted HRs of the Q4 of TSAT were 0.76 (0.62-0.93) for CVD mortality and 1.47 (1.07-2.03) for cancer mortality.

Conclusion: Iron metabolism abnormalities increase mortality risk in the general population. The associations of iron status with mortality were significantly different between individuals with and without DM, which indicated tailored strategies for iron homeostasis are needed.

Key Words: iron metabolic biomarkers, mortality, diabetes mellitus

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA_{1c}, glycated hemoglobin, A_{1c}; HR, hazard ratio; ICD-10, International Classification of Diseases, Tenth Revision; ID, iron deficiency; NHANES, National Health and Nutrition Examination Survey; Q, quartile; ROS, reactive oxygen species; SF, serum ferritin; TIBC, total iron-binding capacity; TSAT, transferrin saturation; WBC, white blood cell count.

Iron is an essential element in the human body and plays a critical role in many physiological and cellular processes [1]. Dysregulated iron homeostasis could result in several pathological problems. Iron deficiency (ID), irrespective of concomitant anemia, affects various systems, including cognitive impairments, weakened immune system, hemodynamic instability, etc [2, 3]. Excess iron, leading to subsequent deposition in vital organs or iron-related oxidative stress over time, increases the risk of cirrhosis, atherosclerosis, diabetes, cancer, and heart failure [4-7]. In addition, iron metabolic disorders have been identified as strong predictors of mortality in patients with certain diseases, such as heart failure, chronic kidney disease, and hemochromatosis [3, 8, 9]. In the general population, obesity, chronic inflammation, and unhealthy lifestyles can increase the risk of iron metabolic disorders [10]. Thus, understanding the association of iron metabolic biomarkers with the long-term prognosis in the general population is particularly important to screen for people at high risk of death.

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Serum ferritin (SF) and transferrin saturation (TSAT) are 2 commonly measured biomarkers of iron metabolism in clinical settings [11], which reflects iron storage [12] and iron availability, respectively [13]. The abnormal range of SF and TSAT are used to indicate iron metabolism abnormalities [14]. Previous epidemiologic studies on the association between iron status and the risk of all-cause or cause-specific mortality in different cohorts have provided conflicting results [15-18]. Most studies evaluated the association between a single biomarker of iron metabolism and the risk of mortality in a limited number of individuals, which may not reflect the full complexity of iron metabolism and its effect on health outcomes [19-21]. Of note, iron is involved in insulin production and glucose metabolism. It has been reported that iron metabolic disorders have detrimental effects on insulin secretion and insulin sensitivity, which consequently affects the long-term prognosis among those with diabetes mellitus (DM) [22-24]. However, it is currently unclear whether the association between iron metabolic biomarkers and the risk of mortality differs between people with and without DM.

Accordingly, using a large sample from a nationally representative cohort, our study aimed to investigate the association between iron metabolic biomarkers and the risk of all-cause and cause-specific mortality in a general population in the United States, as well as evaluate the potential heterogeneity in this association between individuals with and without DM.

Materials and Methods

Study Population

We performed a population-based cohort study using data collected prospectively from the National Health and Nutrition Examination Survey (NHANES). NHANES is a research program designed to assess the health and nutritional status of adults and children in the United States, using stratified, multistage probability sampling. More detailed information about the NHANES survey design and the NHANES database can be accessed publicly at https://www.cdc.gov/nchs/nhanes/index.htm. Our analysis was conducted on the data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) and 6 subsequent cycles of NHANES (1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, and 2009-2010). In NHANES, participants were not necessarily the same individuals in each of the surveys, therefore, all participants were recruited once with a unique sequence number. The study protocol for NHANES was approved by the National Center for Health Statistics Research ethics review board. All participants provided written informed consent. This study was based on secondary analyses of publicly available and deidentified NHANES data. Therefore, no further institutional review board approval was needed for this study.

All participants aged 20 years or older with an SF or TAST measurement at baseline were enrolled in our analysis. We then excluded those meeting any of the following criteria: (1) pregnant women (n = 1037); (2) participants having a malignant tumor (n = 2776); or (3) loss of follow-up (n = 26). Finally, 29 166 participants were included in the analysis of SF, of whom 3846 had DM. A total of 26 390 participants were included in the analysis of TSAT, of whom 3621 had DM (Supplementary Fig. S1) [25].

Measurements of Serum Ferritin and Transferrin Saturation

In NHANES III and NHANES 1999 to 2003, measurement of SF was performed using the BioRad method, and the Hitachi (Roche) method was used to measure SF in NHANES 2004 to 2010. To standardize the ferritin data measured using BioRad for comparison with the Hitachi (Roche) method data, we employed the following piecewise linear regression equations:

$$SF \leq 25: Y(\text{Hitachi}) = 1.2534 * X(\text{BioRad}) + 1.4683$$

$$25 < SF \leq 65: Y(\text{Hitachi}) = 1.2001 * X(\text{BioRad}) + 1.4693$$

$$SF > 65: Y(\text{Hitachi}) = 1.0791 * X(\text{BioRad}) + 4.8183.$$

The TSAT value was calculated as (iron/TIBC) × 100%. Serum iron and total iron-binding capacity (TIBC) are measured by a modification of the automated AAII-25 colorimetric method. A detailed description of the laboratory method used and data standardization can be found on the NHANES website [26, 27].

Data Collection and Definition

Data on demographic characteristics, socioeconomic indicators, and self-reported diseases were collected through standardized questionnaires. Demographic characteristics included age, sex (male, female), and race/ethnicity (Mexican American, Non-Hispanic White, Non-Hispanic Black, and Other Race). Socioeconomic indicators included education level (lower than high school, high school, higher than high school, and unknown), family income, and occupation (unemployed, employed, and unknown). Family income level was categorized into 3 groups: low (≤1), middle (2-3), and high (≥4) based on the poverty-to-income ratio. The employed group of occupation was defined as those with a job or business, retired, or students. Self-reported diseases included hypertension and cardiovascular disease (CVD). Self-reported CVD consisted of angina/angina pectoris, heart attack, and stroke. Menopausal status for women was assessed based on the question from the NHANES Reproductive Health Questionnaire: "What is the reason that you have not had a period in the past 12 months?" Participants who answered "menopause" or "hysterectomy" were categorized as being in the postmenopausal group. DM was defined when participants met one of the following criteria: (1) self-reported diagnosis of diabetes; (2) glycated hemoglobin A_{1c} level (HbA_{1c}) greater than or equal to 6.5%; (3) fasting blood glucose greater than or equal to 7.0 mmol/L; (4) postprandial 2-hour plasma glucose level greater than or equal to 11.1 mmol/L from an oral glucose tolerance test [28]; or (5) currently using insulin or taking antidiabetic medications.

Physical examinations in the NHANES study were conducted in a controlled environment called the mobile examination center with consistent measurements. Body mass index (BMI) was captured electronically from the measuring instruments in the mobile examination center to minimize potential data entry errors. BMI was categorized as underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), and obese (≥30.0) [29].

Blood samples were collected, processed, and transported to NHANES analytical laboratories following validated

procedures. Data for the laboratory component were recorded directly into a computerized database and integrated with the main NHANES survey database. Laboratory data on glycated hemoglobin (HbA_{1c}, %), creatinine (mg/dL), alanine transaminase (ALT, U/L), aspartate transaminase (AST, U/L), hemoglobin (Hb, g/dL), and white blood cell count (WBC) were acquired in all cycles. The estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) was calculated from the serum creatinine [30].

Ascertainment of Outcomes

All-cause and cause-specific mortalities were ascertained by the National Death Index (NDI), which is a database of all deaths in the United States, with a unique sequence number in the National Center for Health Statistics of the United States through December 31, 2019 [31]. All events were coded using the International Classification of Diseases (ICD), 10th revision (ICD-10), including all-cause, CVD (I00-I09, I11, I13, I20-I51, I60-I69), cancer (C00-C97) mortality [32]. The follow-up time was defined from baseline to death or the end of follow-up (December 31, 2019).

Statistical Analyses

Baseline characteristics were described according to the presence of DM. Continuous variables were described by mean SD or median with interquartile range, and categorical variables were described by frequency with percentage. Baseline characteristics were compared using the 2 independent-sample *t* test or Wilcoxon test for continuous variables, and the chi-square test for categorical variables.

We categorized participants into quartiles according to baseline SF and TSAT levels (details showed in Supplementary Table S1 [25]), and used Cox proportional-hazards models to estimate hazard ratios (HRs) and 95% CIs for the risk of all-cause mortality. The proportional hazard assumption was tested based on Schoenfeld residuals and no violation was found. For CVD and cancer mortality, we performed Fine-Gray analysis with death as competing risk. In all models, quartile 1 (Q1) for each indicator was used as the reference. All the analyses were conducted in the general population and separately among individuals with and without DM. We fitted 3 different models with an increasing number of potential confounders. Model 1 was adjusted for age (continuous), sex (male and female), and race/ethnicity (Mexican American, Non-Hispanic Black, Non-Hispanic White, and other). Model 2 was further adjusted for education level (lower than high school, high school, higher than high school, and unknown), family income (low, middle, high, and unknown), and occupation (employed, unemployed, and unknown). Finally, model 3 was the same as model 2 plus the following additional variables: BMI (underweight, normal, overweight, and obese), WBC, ALT, AST, eGFR, and medical history (self-reported hypertension and CVD). In addition, we fitted models with an interaction term to evaluate the interaction between DM and iron status. Furthermore, 3 sensitivity analyses were conducted to test the robustness of the results. First, we excluded participants whose outcome occurred within the first year of follow-up to avoid reverse causality. Second, sex-stratified analyses were performed, and menopausal status was incorporated in the analysis for women. Third, we performed analyses including deaths that occurred in the first 3 and 10 years during the follow-up, respectively.

To examine the dose-response relationship between iron status biomarkers and outcomes, restricted cubic spline regression models with 3 knots (10th, 50th, and 90th percentiles) were employed based on Cox proportional-hazards models. Tests for nonlinearity were performed using the likelihood ratio test.

To minimize sample size reduction due to missing covariates, we imputed the missing values of continuous covariates (≤2%) using multiple imputations. A 2-sided *P* value of less than .05 was considered statistically significant and all statistical analysis was performed using SAS version 9.4 (SAS Institute).

Results

Baseline Characteristics

Baseline characteristics of participants are shown in Table 1. Among 29 166 participants from NHANES (mean age 45.9 years, 59.9% female), 3846 (13.19%) had DM. The median (interquartile range) of SF was 77 ng/mL (35-157 ng/mL) and TAST was 23.4% (17.1%-31.1%) in the general population. Compared to those without DM, those with DM were more likely to be older, male, Mexican American or non-Hispanic Black, with lower education level and lower family income ($P < .001$). They also had a higher prevalence of obesity and comorbidities ($P < .001$). Participants with DM had higher levels of SF, but lower levels of TSAT ($P < .001$). Additionally, individuals with DM had higher levels of WBC, AST, ALT, Hb, and HbA_{1c}, but lower levels of eGFR (all $P < .001$).

Association Between Serum Ferritin and All-Cause and Cause-Specific Mortality

A total of 9378 deaths (16.96/1000 person-years) were observed during a median follow-up duration of 18.83 years, including 3420 (6.18/1000 person-years) CVD deaths and 1969 (3.56/1000 person-years) cancer deaths. Among the population with DM, there were 2493 (42.35/1000 person-years) all-cause deaths, including 968 (16.44/1000 person-years) CVD deaths and 385 (6.54/1000 person-years) cancer deaths. In the population without DM, there were 6885 (13.87/1000 person-years) all-cause deaths, including 2452 (4.96/1000 person-years) CVD deaths and 1584 (3.21/1000 person-years) cancer deaths.

Significant linear associations between SF levels and all-cause, and CVD mortality were observed among the overall population and those without DM. In people without DM, a significant linear association between SF levels and all-cause mortality was observed (all *P* for overall <.05 and *P* for nonlinearity >.05). In people with DM, linear associations were also observed between SF levels and all-cause and CVD mortality, but they were not statistically significant (*P* for overall >.05 and *P* for nonlinearity >.05) (Fig. 1). In the analysis of SF quartiles, in the overall population, compared to people in the Q1 group, the HR (95% CI) for all-cause mortality after adjusting for all covariates (model 3) was 1.07 (1.00-1.15), 1.05 (0.98-1.12), and 1.13 (1.05-1.21) in the Q2, Q3, and Q4 groups, respectively. The adjusted HRs (95% CIs) of the Q4 were 1.13 (1.01-1.28) for CVD mortality and 1.23 (1.05-1.43) for cancer mortality, respectively (Table 2). In the sensitivity analyses, the results were not materially changed in the sensitivity analyses (Supplementary Fig. S2 and Supplementary Tables S2-S6) [25]. A significantly

Table 1. Baseline characteristics of participants stratified by diabetes in NHANES III and NHANES 1999 to 2010

	Total	Without diabetes	With diabetes	*P*
Participants	29 166	25 320	3846	
Age, y	45.9 ± 18.9	43.8 ± 18.6	59.5 ± 15.1	<.001
Female	17 465 (59.9)	15 277 (60.3)	2188 (56.9)	<.001
Race				<.001
Mexican American	7504 (25.7)	6361 (25.1)	1143 (29.7)	
Non-Hispanic Black	7041 (24.1)	6021 (23.8)	1020 (26.5)	
Non-Hispanic White	12 631 (43.3)	11 188 (44.2)	1443 (37.5)	
Other	1990 (6.8)	1750 (6.9)	240 (6.2)	
Education level				<.001
Lower than high school	7813 (26.79)	6191 (24.5)	1622 (42.2)	
High school	10 807 (37.1)	9444 (37.3)	1363 (35.4)	
Higher than high school	10 411 (35.7)	9565 (37.8)	846 (22.0)	
Unknown	135 (0.5)	120 (0.5)	15 (0.4)	
Family income				<.001
Low	6023 (20.7)	5110 (20.2)	913 (23.7)	
Middle	15 018 (51.5)	13 008 (51.4)	2010 (52.3)	
High	5422 (18.6)	4931 (19.5)	491 (12.8)	
Unknown	2703 (9.3)	2271 (9.0)	432 (11.2)	
Occupation				<.001
Employed	18 685 (64.1)	16 949 (66.9)	1736 (45.1)	
Unemployed	3061 (10.5)	2648 (10.5)	413 (10.7)	
Unknown	7420 (25.4)	5723 (22.6)	1697 (44.1)	
Body mass index				<.001
Underweight	670 (2.3)	630 (2.5)	40 (1.0)	
Normal weight	10 256 (35.2)	9553 (37.7)	703 (18.3)	
Overweight	9948 (34.1)	8581 (33.9)	1367 (35.5)	
Obese	8292 (28.4)	6556 (25.9)	1736 (45.1)	
Self-reported hypertension	7466 (25.6)	5494 (21.7)	1972 (51.3)	<.001
Self-reported CVD	1957 (6.7)	1311 (5.2)	646 (16.8)	<.001
White blood cell count	7.0 (5.7-8.4)	6.9 (5.7-8.3)	7.5 (6.1-8.9)	<.001
Alanine aminotransferase, U/L	17.0 (12.0-24.0)	16.0 (12.0-23.0)	17.0 (12.0-25.0)	<.001
Aspartate transaminase, U/L	20.0 (17.0-25.0)	20.0 (17.0-25.0)	20.0 (17.0-26.0)	<.001
eGFR, mL/min/1.73 m^2	104.4 (88.6-115.9)	106.3 (92.0-117.1)	88.2 (69.3-103.5)	<.001
Hemoglobin, g/dL	13.9 (13.0-15.0)	13.9 (13.0-15.0)	14.0 (12.9-15.0)	<.001
Glycated hemoglobin, %	5.3 (5.0-5.7)	5.3 (5.0-5.5)	6.7 (5.9-8.3)	<.001
Serum ferritin, ng/mL	77.0 (35.0-157.0)	72.0 (33.0, 145.0)	125.0 (59.0-243.0)	<.001
Transferrin saturation, %	23.4 (17.1-31.1)	23.6 (17.3-31.4)	21.9 (16.4-28.9)	<.001

Abbreviations: CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; NHANES, National Health and Nutrition Examination Survey.

different association between those with and without DM was found for CVD and cancer mortality, but not for all-cause mortality (Supplementary Table S7) [25]. There was no significant association between SF quartiles and all-cause or cause-specific mortality in those with DM. In contrast, in individuals without DM, the adjusted HRs (95% CIs) of the Q4 were 1.19 (1.03-1.37) for CVD mortality and 1.25 (1.05-1.48) for cancer mortality, respectively (see Table 2).

Association Between Transferrin Saturation and All-Cause and Cause-Specific Mortality

We observed J-shaped associations between TSAT and mortality in the general population and people with or without DM. The nonlinear associations were significant for all-cause and CVD mortality (*P* for overall <.05 and *P* for nonlinearity <.05), while the linear associations were not significant for cancer mortality (*P* for overall >.05 and *P* for nonlinearity >.05). In the lower range of TSAT (<20%), TSAT levels were negatively associated with the risk of all-cause and CVD mortality, while very high TSAT (>50%) was also associated with an increased risk of all-cause and CVD mortality (Fig. 2). In the analysis of TSAT quartiles, in the overall population, compared to the Q1 group, the HR (95% CI) for all-cause mortality after adjusting for all covariates (model 3) was 0.94 (0.88-0.99), 0.92 (0.86-0.97), and 0.93 (0.88-0.99) in the Q2, Q3, and Q4 groups, respectively. The adjusted HRs (95% CIs) of the Q4 were 0.86 (0.77-0.96)

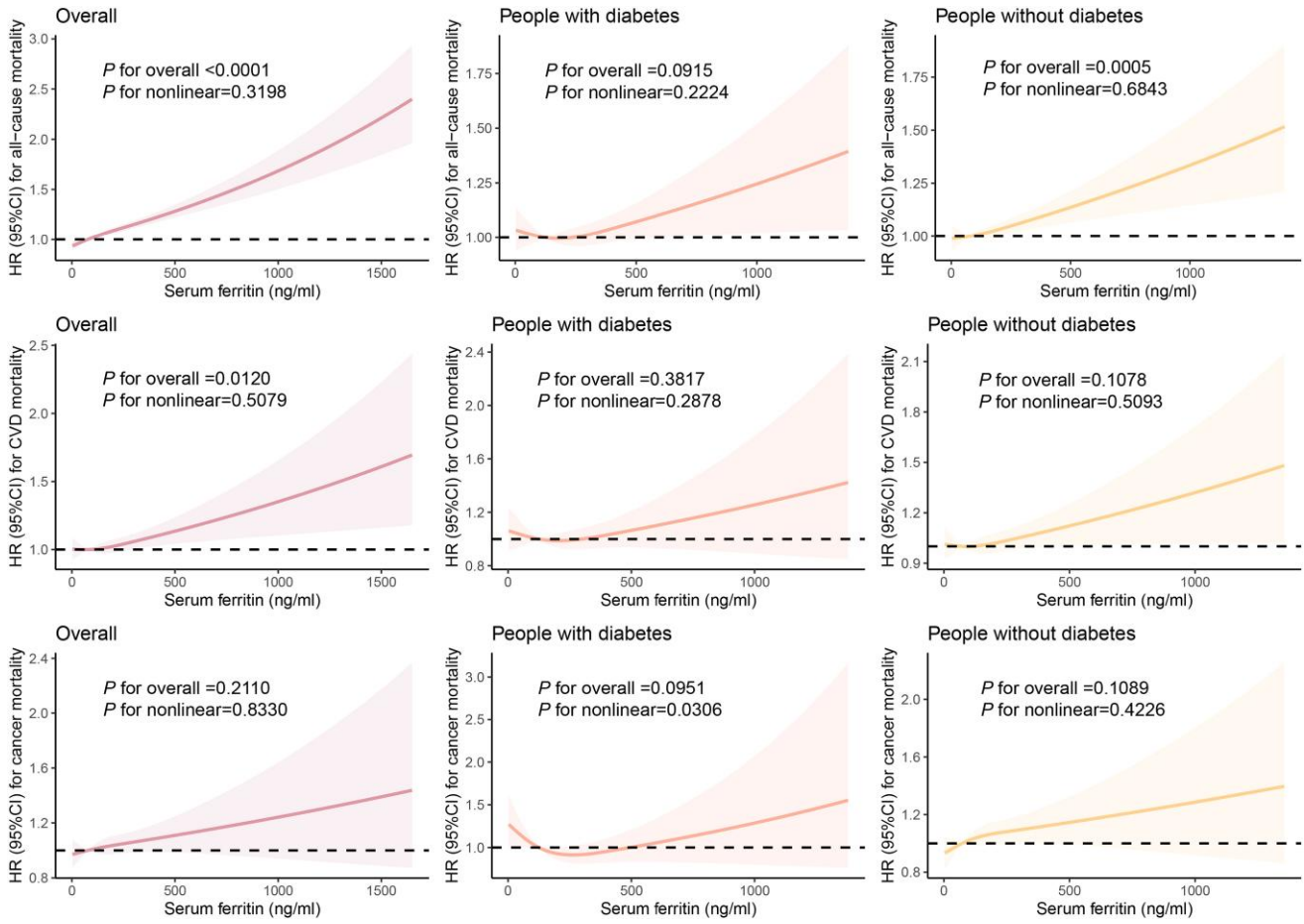

Figure 1. Association relationships between serum ferritin (SF) and risk of all-cause and cause-specific mortality. We used restricted cubic splines (RCS) incorporated in the Cox models with 3 predefined knots at the 10th, 50th, and 90th centiles to evaluate the association relationships between SF and risk of mortality. All models were adjusted for age, sex, race, education, family income, occupation, body mass index, white blood cell count, alanine transaminase, aspartate transaminase, estimated glomerular filtration rate, hypertension, and cardiovascular disease.

for CVD mortality and 1.14 (0.99-1.31) for cancer mortality, respectively (Table 3). In the sensitivity analyses, the results were not materially changed in the sensitivity analyses (Supplementary Fig. S3 and Supplementary Tables S8-S12) [25]. No significantly different association between those with and without DM was found for all outcomes (Supplementary Table S13). Among individuals with DM, there was no significant association between TSAT quartiles and all-cause mortality. Compared to the Q1 group, the adjusted HRs (95% CIs) of the Q4 were 0.76 (0.62-0.93) for CVD mortality and 1.47(1.07-2.03) for cancer mortality. Among individuals without DM, the adjusted HRs (95% CIs) of the Q3 of TAST were 0.91 (0.85-0.98) for all-cause mortality, but there was no significant association between TSAT quartiles and CVD or cancer mortality (see Table 3).

Discussion

Main Findings

This study is the largest and most comprehensive study to date that estimates the association of iron status including SF and TSAT with the risk of all-cause and cause-specific mortality in a general population. We identified that iron metabolic abnormalities were associated with an increased risk of mortality in the general population. Specifically, high iron storage indicated by higher SF was associated with an increased risk of all-cause and cause-specific mortality. ID, presented by lower TSAT levels, was associated with an increased risk of all-cause and CVD mortality. Furthermore, significant differences in the association of iron status with mortality between individuals with and without DM were identified. Among those without DM, higher SF levels were significantly associated with an increased risk of all-cause and cause-specific mortality. In individuals with DM, lower TSAT levels were significantly associated with an increased risk of CVD mortality, but a reduced risk of cancer mortality.

High Iron Status and Mortality Risk

In our study, high iron status, primarily indicated by elevated levels of SF, was found to be associated with an increased risk of all-cause and cause-specific mortality in the general population. Participants in the highest quartile of SF (>157 ng/mL) had a 13% higher risk of all-cause mortality, a 19% higher risk of CVD mortality, and a 23% higher risk of cancer mortality compared to those in the lowest quartile of SF (<35 ng/mL). Our findings align with a Danish population-based study concluding that the moderately to markedly increased SF levels represented a biological biomarker predictive of early death in a dose-dependent linear manner in the general population. Additionally, the Danish study revealed that markedly

Table 2. Hazard ratios (95% CIs) for mortality according to quartiles of serum ferritin among individuals in NHANES III and NHANES 1999 to 2010

	Serum ferritin, ng/mL			
	Q1	**Q2**	**Q3**	**Q4**
Overall				
No. of total	7448	7149	7326	7243
Range	0-35	35-77	77-157	>157
All-cause mortality				
No. of deaths	1282	1998	2658	3440
Model 1	1 (reference)	1.05 (0.98-1.12)	1.03 (0.96-1.10)	1.12 (1.05-1.20)
Model 2	1 (reference)	1.06 (0.99-1.14)	1.03 (0.96-1.10)	1.12 (1.05-1.20)
Model 3	1 (reference)	1.07 (1.00-1.15)	1.05 (0.98-1.12)	1.13 (1.05-1.21)
CVD mortality				
No. of deaths	477	740	959	1244
Model 1	1 (reference)	1.03 (0.92-1.17)	1.02 (0.92-1.15)	1.12 (1.00-1.25)
Model 2	1 (reference)	1.05 (0.93-1.18)	1.03 (0.91-1.16)	1.12 (1.00-1.26)
Model 3	1 (reference)	1.05 (0.93-1.19)	1.03 (0.91-1.16)	1.13 (1.01-1.28)
Cancer mortality				
No. of deaths	274	416	561	718
Model 1	1 (reference)	1.13 (0.97-1.33)	1.17 (1.00-1.36)	1.23 (1.06-1.44)
Model 2	1 (reference)	1.14 (0.97-1.33)	1.17 (1.00-1.36)	1.23 (1.06-1.43)
Model 3	1 (reference)	1.14 (0.98-1.34)	1.18 (1.01-1.37)	1.23 (1.05-1.43)
With diabetes				
No. of total	970	957	964	955
Range	0-59	59-125	125-243	>243
All-cause mortality				
No. of deaths	512	634	661	686
Model 1	1 (reference)	0.99 (0.88-1.11)	0.97 (0.87-1.10)	1.03 (0.92-1.16)
Model 2	1 (reference)	1.00 (0.89-1.12)	0.96 (0.86-1.08)	1.02 (0.91-1.15)
Model 3	1 (reference)	1.02 (0.91-1.15)	0.99 (0.88-1.11)	1.05 (0.93-1.19)
CVD mortality				
No. of deaths	211	239	271	247
Model 1	1 (reference)	0.90 (0.75, 1.09)	0.99 (0.83, 1.20)	0.90 (0.74, 1.09)
Model 2	1 (reference)	0.90 (0.75-1.10)	0.99 (0.82-1.19)	0.89 (0.73-1.08)
Model 3	1 (reference)	0.93 (0.76-1.12)	1.03 (0.85-1.24)	0.97 (0.79-1.19)
Cancer mortality				
No. of deaths	83	109	94	99
Model 1	1 (reference)	1.13 (0.84-1.51)	0.91 (0.67-1.23)	0.96 (0.70-1.31)
Model 2	1 (reference)	1.11 (0.83-1.49)	0.89 (0.66-1.21)	0.96 (0.70-1.30)
Model 3	1 (reference)	1.10 (0.82-1.47)	0.85 (0.63-1.15)	0.89 (0.65-1.23)
Without diabetes				
No. of total	6471	6199	6336	6314
Range	0-33	33-72	72-145	>145
All-cause mortality				
No. of deaths	938	1452	1926	2569
Model 1	1 (reference)	1.03 (0.94-1.11)	0.99 (0.92-1.08)	1.05 (0.97-1.14)
Model 2	1 (reference)	1.03 (0.95-1.12)	0.99 (0.92-1.08)	1.05 (0.97-1.14)
Model 3	1 (reference)	1.02 (0.94-1.11)	1.01 (0.90-1.12)	1.06 (0.98-1.15)
CVD mortality				
No. of deaths	317	549	694	892
Model 1	1 (reference)	1.16 (1.00, 1.34)	1.14 (0.99, 1.31)	1.14 (0.99, 1.31)
Model 2	1 (reference)	1.18 (1.02, 1.36)	1.15 (0.99, 1.32)	1.15 (1.00, 1.32)
Model 3	1 (reference)	1.20 (1.03-1.39)	1.15 (1.00-1.33)	1.19 (1.03-1.37)

(*continued*)

Table 2. Continued

	Serum ferritin, ng/mL			
	Q1	Q2	Q3	Q4
Cancer mortality				
No. of deaths	225	303	442	614
Model 1	1 (reference)	1.01 (0.84-1.21)	1.11 (0.94-1.32)	1.22 (1.03-1.44)
Model 2	1 (reference)	1.01 (0.85-1.21)	1.12 (0.94-1.32)	1.22 (1.03-1.45)
Model 3	1 (reference)	1.02 (0.85-1.22)	1.13 (0.96-1.35)	1.25 (1.05-1.48)

Cox proportional-hazards models adjusted for model 1: age, sex, and race (Mexican American, Non-Hispanic Black, Non-Hispanic White, other); model 2: plus education (lower than high school, high school, higher than high school, and unknown), family income (low, middle, high, and unknown), and occupation (employed, unemployed, and unknown); model 3: plus body mass index (underweight, normal, overweight, obese), white blood cell count, alanine transaminase, aspartate transaminase, estimated glomerular filtration rate, and medical history (hypertension and CVD).
Abbreviations: CVD, cardiovascular disease; NHANES, National Health and Nutrition Examination Survey; Q, quartile.

increased SF levels are associated with a 50% higher risk of total mortality, a 50% higher risk of CVD mortality, and a 60% higher risk of cancer mortality compared to the group with the lowest SF concentrations [19]. However, previous studies based on NHANES III and II did not observe a significant association between SF levels and risk of all causes, CVD, or cancer mortality, which may be caused by limited sample size or short duration of follow-up [16, 17]. In our study, the J-shaped curve relationship between TSAT and mortality indicated that very high TSAT levels (>50%) were associated with a higher risk of all-cause and CVD mortality, which is consistent with previous studies [21, 33].

High iron damage is believed to occur due to an imbalance in the iron and ferritin-transferrin control system [14], that is, once the iron burden exceeds the capacity of the ferritin-transferrin control system, it will lead to tissue damage and disease pathology by producing large amounts of reactive oxygen species (ROS) through the Fenton reaction [34]. Excessive ROS production can lead to oxidative stress, causing damage to lipids, proteins, and DNA, which may accumulate over time and cause chronic diseases such as cancer and CVD or death [16].

Iron Deficiency and Mortality Risk

In our study, we observed that ID, mainly shown by low TSAT levels, was associated with an increased risk of all-cause and CVD mortality. Compared with the lowest quartile (<17.1%) of TSAT, the all-cause and CVD mortality risk of the highest quartile (>31.1%) was reduced by 7% and 14%, respectively. Meanwhile, the relationship between SF levels and risk of all-cause and cause-specific mortality was linear, and it is not possible to determine specific thresholds for increased risk of death due to ID based on SF levels. Our findings are consistent with a European population-based study showing that functional iron deficiency complemented by a low TSAT (<20%) was associated with CV death and all-cause death [35]. The potential mechanism of ID's effect, especially low iron availability, on health is that ID hinders basic physiological functions that rely on iron, such as erythropoiesis, oxygen transport and storage, mitochondrial function, and synthesis and degradation of proteins, lipids, and ribonucleic acids, as well as myocardial and skeletal muscle metabolism, which leads to a worse clinical outcome [1]. Additionally, cardiomyocytes and other cells with high energy demands are especially vulnerable to the effects of ID on metabolism and cellular energetics [36].

Previous guidelines have recommended the measurement of SF concentrations, and some proposed TSAT as an alternative or complementary to define ID, that is SF less than 100 g/mL or 100 to 299 ng/mL with TSAT less than 20% [3]. However, some recent studies have realized that TSAT is a reliable diagnostic maker for ID with high sensitivity and specificity and should be recommended in clinical practice and a novel definition of ID based on TSAT alone has been suggested [37]. In a pulmonary vascular disease study, researchers found that ID, defined by TSAT less than 21%, best identifies patients with worse functional status and adverse clinical outcomes [38]. Our results underscored that ID, indicated by a lower level of TSAT, was associated with a higher risk of mortality. Clinical practice should consider low TSAT when evaluating ID and estimating patients' prognosis. This variation in TSAT thresholds may be attributed to various factors, including the heterogeneity of the study population and study area, as well as the testing instruments, reagents, and methods.

Differences Between Individuals With and Without Diabetes

We found the association between iron status biomarkers and mortality risk varies between individuals with and without diabetes. First, individuals with DM had higher levels of SF than those without DM, but the association between SF levels and risk of mortality was not significant in those with DM. In addition to serving as a marker for iron storage, SF is also influenced by acute or chronic inflammatory states, liver disease, obesity, and insulin resistance, which are common among patients with DM [39]. Consistent with baseline characteristics of our study population, individuals with DM had higher levels of BMI, WBC, AST, and ALT than those without DM. Thus, higher SF levels in DM individuals may be caused by inflammation or other diabetes-related mechanisms rather than by high iron stores [40]. Second, individuals with DM had lower levels of TSAT compared to those without DM, and the Q1 group of TSAT was associated with higher CVD mortality risk and lower cancer mortality risk. TSAT reflects the proportion of circulating iron in the context of iron requirements and is less affected by inflammatory processes [37, 39, 40], which means the ID was more pronounced in individuals with diabetes leading to cardiomyocyte energy metabolism disorders, and increased risk of CVD mortality. In

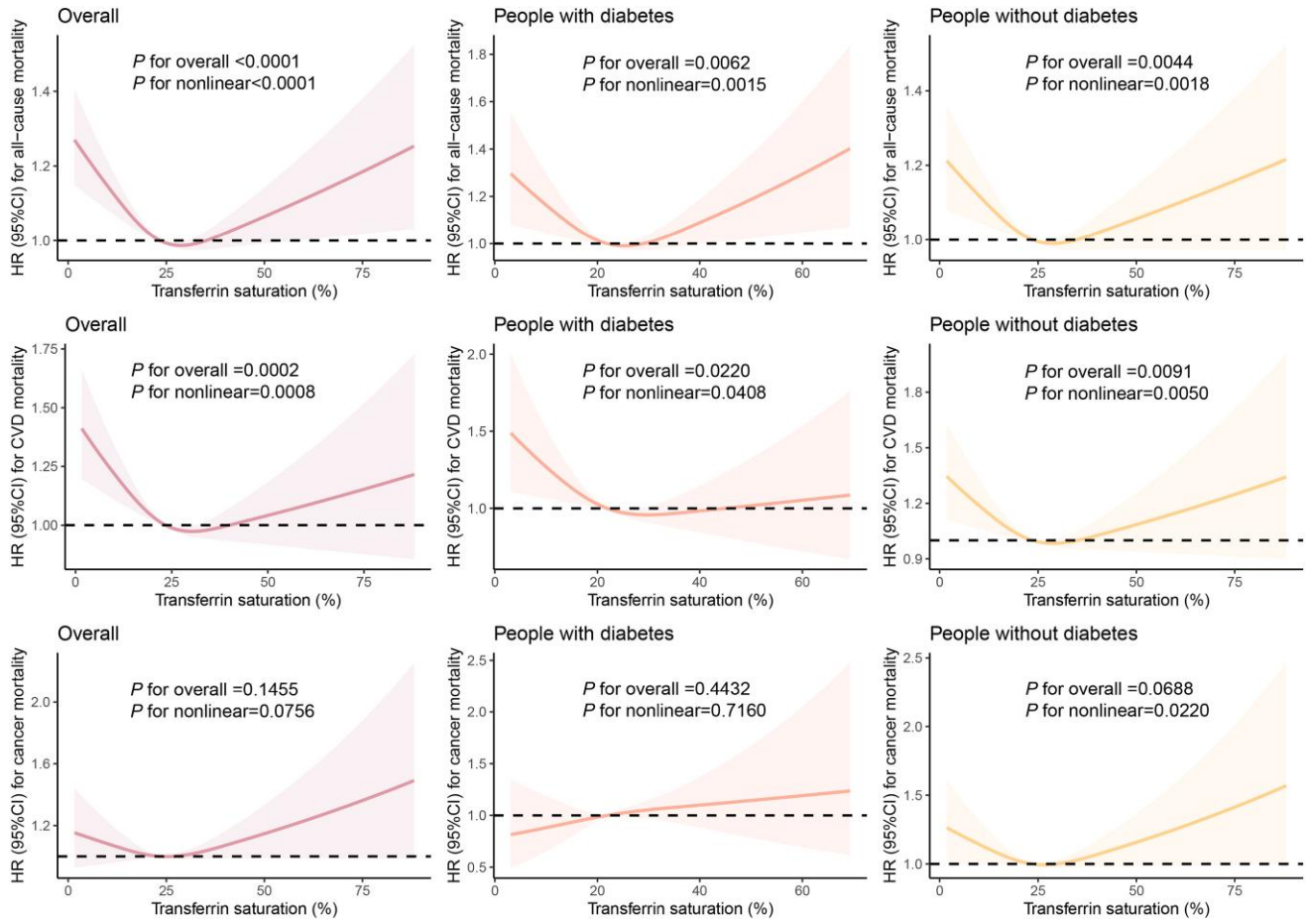

Figure 2. Association relationships between transferrin saturation (TSAT) and risk of all-cause and cause-specific mortality. We used restricted cubic splines (RCS) incorporated in the Cox models with 3 predefined knots at the 10th, 50th, and 90th centiles to evaluate the association relationships between transferrin saturation and risk of mortality. All models were adjusted for age, sex, race, education, family income, occupation, body mass index, white blood cell count, alanine transaminase, aspartate transaminase, estimated glomerular filtration rate, hypertension, and cardiovascular disease.

addition to its role in the generation of ROS, iron may be a limiting nutrient to the growth and replication of cancer cells in the human body, which may be responsible for the lowest cancer mortality risk when iron is deficient [15].

We need to highlight one unanticipated finding in the DM population. The increased availability of iron had the opposite effect on the risk of CVD and cancer mortality, that is, higher TSAT was significantly positively associated with CVD risk but significantly inversely associated with cancer mortality risk. This poses a great challenge for clinical treatment. Intravenous iron supplementation has been demonstrated in previous clinical studies to specifically improve symptoms, health-related quality of life, exercise capacity, and reduce hospitalizations in CVD patients [1, 41], but whether it increases cancer mortality risk has not been included as an end point in previous studies. This study suggests that we must exercise caution when administering iron supplements to CVD patients complicated by DM, and future clinical studies are necessary to evaluate the potential risk of cancer mortality associated with the use of iron supplements.

Clinical Importance

Our study has potential implications for clinical practice and health promotion. First, the comprehensive assessment of iron status and mortality in this study could have implications for interpreting SF and TAST levels in clinical practice and for predicting mortality risk based on whether individuals have DM. Second, for nutritionists and clinicians, these findings can guide the development of appropriate management strategies to maintain iron homeostasis in individuals, involving regular monitoring of SF and TSAT levels to assess iron status and providing tailored dietary recommendations, iron supplementation, or iron chelators when necessary. Third, the study provides evidence-based guidance for realistic public health recommendations to promote and maintain iron homeostasis by raising awareness about the risk of ID and high iron status, as well as educating individuals on the importance of regularly monitoring their iron status.

Strengths and Limitations

Our research has some considerable advantages. First, a major strength of this study was the long-term follow-up of a large number of participants from a nationally representative database, providing more solid conclusions. Second, a restricted cubic spline was used to explore the nonlinear relationship between iron status indices and all end points. However, there are several limitations in the present study that need to be addressed. First, the use of self-reported chronic diseases may introduce recall bias and may not accurately reflect the true health status of the participants. Second, the study included

Table 3. Hazard ratios (95% CIs) for mortality according to quartiles of transferrin saturation among individuals in NHANES III and NHANES 1999 to 2010

	Transferrin saturation, %			
	Q1	**Q2**	**Q3**	**Q4**
Overall				
No. of total	6657	6613	6569	6551
Range	0-17.1	17.1-23.4	23.4-31.1	>31.1
All-cause mortality				
No. of deaths	2173	2537	2362	2215
Model 1	1 (reference)	0.90 (0.85-0.96)	0.84 (0.79-0.89)	0.87 (0.82-0.92)
Model 2	1 (reference)	0.91 (0.86-0.96)	0.86 (0.81-0.92)	0.88 (0.82-0.93)
Model 3	1 (reference)	0.94 (0.88-0.99)	0.92 (0.86-0.97)	0.93 (0.88-0.99)
CVD mortality				
No. of deaths	850	962	859	728
Model 1	1 (reference)	0.93 (0.85-1.03)	0.86 (0.77-0.95)	0.80 (0.72-0.89)
Model 2	1 (reference)	0.94 (0.85-1.04)	0.87 (0.79-0.96)	0.80 (0.72-0.89)
Model 3	1 (reference)	0.96 (0.86-1.06)	0.92 (0.83-1.02)	0.86 (0.77-0.96)
Cancer mortality				
No. of deaths	424	522	490	510
Model 1	1 (reference)	1.05 (0.92-1.20)	0.99 (0.86-1.13)	1.10 (0.96-1.26)
Model 2	1 (reference)	1.05 (0.92-1.20)	1.00 (0.87-1.14)	1.10 (0.96-1.26)
Model 3	1 (reference)	1.09 (0.95-1.24)	1.03 (0.90-1.18)	1.14 (0.99-1.31)
With diabetes				
No. of total	918	902	903	898
Range	0-16.4	16.4-21.9	21.9-28.9	>28.9
All-cause mortality				
No. of deaths	583	624	628	632
Model 1	1 (reference)	0.88 (0.79-0.99)	0.89 (0.79-0.99)	0.90 (0.81-1.02)
Model 2	1 (reference)	0.88 (0.79-0.99)	0.90 (0.80-1.01)	0.91 (0.81-1.02)
Model 3	1 (reference)	0.90 (0.80-1.01)	0.95 (0.84-1.06)	0.94 (0.83-1.06)
CVD mortality				
No. of deaths	249	239	254	215
Model 1	1 (reference)	0.86 (0.72-1.04)	0.90 (0.75-1.08)	0.71 (0.59-0.87)
Model 2	1 (reference)	0.85 (0.71-1.03)	0.90 (0.75-1.08)	0.71 (0.58-0.87)
Model 3	1 (reference)	0.86 (0.72-1.04)	0.93 (0.77-1.13)	0.76 (0.62-0.93)
Cancer mortality				
No. of deaths	68	103	104	105
Model 1	1 (reference)	1.45 (1.07-1.98)	1.46 (1.07-1.99)	1.47 (1.07-2.00)
Model 2	1 (reference)	1.44 (1.06-1.97)	1.46 (1.07-1.99)	1.45 (1.06-1.98)
Model 3	1 (reference)	1.47 (1.08-2.02)	1.46 (1.07-1.99)	1.47 (1.07-2.03)
Without diabetes				
No. of total	5756	5639	5705	5669
Range	0-17.3	17.3-23.6	23.6-31.4	>31.4
All-cause mortality				
No. of deaths	1545	1849	1757	1669
Model 1	1 (reference)	0.92 (0.86-0.99)	0.85 (0.79-0.91)	0.89 (0.83-0.96)
Model 2	1 (reference)	0.93 (0.87-0.99)	0.87 (0.81-0.93)	0.90 (0.84-0.97)
Model 3	1 (reference)	0.95 (0.89-1.02)	0.91 (0.85-0.98)	0.95 (0.88-1.02)
CVD mortality				
No. of deaths	586	694	623	539
Model 1	1 (reference)	0.96 (0.85-1.08)	0.88 (0.78-0.99)	0.85 (0.75-0.96)
Model 2	1 (reference)	0.97 (0.86-1.09)	0.89 (0.79-1.01)	0.86 (0.75-0.97)

(*continued*)

Table 3. Continued

	Transferrin saturation, %			
	Q1	Q2	Q3	Q4
Model 3	1 (reference)	0.98 (0.87-1.10)	0.93 (0.83-1.05)	0.92 (0.81-1.05)
Cancer mortality				
No. of deaths	346	414	390	416
Model 1	1 (reference)	1.01 (0.87-1.17)	0.92 (0.79-1.07)	1.06 (0.91-1.23)
Model 2	1 (reference)	1.01 (0.87-1.17)	0.93 (0.80-1.08)	1.06 (0.91-1.24)
Model 3	1 (reference)	1.05 (0.90-1.21)	0.97 (0.83-1.13)	1.11 (0.95-1.30)

Cox proportional-hazards models adjusted for model 1: age, sex, and race (Mexican American, Non-Hispanic Black, Non-Hispanic White, and other); model 2: plus education (lower than high school, high school, higher than high school, and unknown), family income (low, middle, high, and unknown), and occupation (employed, unemployed, and unknown); model 3: plus body mass index (underweight, normal, overweight, obese), white blood cell count, alanine transaminase, aspartate transaminase, estimated glomerular filtration rate, and medical history (hypertension and CVD).
Abbreviations: CVD, cardiovascular disease; NHANES, National Health and Nutrition Examination Survey; Q, quartile.

only participants from the United States, which may limit the generalizability of our findings to populations in other countries. Third, the concentration of SF and TSAT was based on a single measurement at baseline and we did not capture the time-varying association of iron status with outcomes. Meanwhile, during almost 20 years of follow-up, the status of DM and confounders will naturally change over time, and we are uncertain about the varying effect of these changes over time. Further research is needed to study the long-term trajectories of iron status indices and their association with outcomes. Fourth, due to the observational design, we could not rule out the unmeasured confounders and assumed causal relationships.

Conclusions

In summary, iron metabolism abnormalities increase the mortality risk in the general population. Higher SF or lower TSAT was associated with an increased risk of all-cause and cause-specific mortality. The associations of iron status with mortality were significantly different between individuals with and without DM. Our findings suggest iron status should be monitored and tailored strategies are needed to promote and maintain iron homeostasis in individuals with and without DM.

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Contributors

J.L., Y.S., and W.P. designed this study. Y.S. and W.P. drafted the manuscript, with further contributions from S.L., J.C., and J.L. Y.S. and W.P. completed all the statistical analysis. All authors interpreted data, contributed to critical revisions, and read and approved the final version of the article.

Disclosures

The authors declare no relevant conflicts of interest.

Data Availability

NHANES data are available at https://www.cdc.gov/nchs/nhanes/index.htm. Mortality data are available at https://www.cdc.gov/nchs/data-linkage/mortality-public.htm.

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