Low Serum Iron Status Is Associated With Low Incidence of Coronary Artery Disease In Women

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Abstract

Background: Disorders of iron metabolism has been implicated in cardiovascular disease. However, the association of serum ferritin and coronary artery disease (CAD) remains inconsistent. Here, we investigated the associations of serum iron metabolism with the incidence of CAD, the severity of coronary artery stenosis, metabolic biomarkers, and 1-year restenosis after coronary artery revascularization.

Methods: A total of 643 CAD patients and 643 healthy controls were enrolled to assess the associations of serum iron status with the presence of CAD, the severity of CAD, and 1-year rehospitalization after revascularization. Serum iron metabolism and other metabolic markers were measured in all subjects. All statistical analyses were analyzed using SPSS22.0 software and STATA statistical package.

Results: Serum level of iron metabolism markers, including serum iron, ferritin, unsaturated transferrin iron binding capacity (UIBC), Total iron binding capacity (TIBC) levels, in CAD groups was significantly higher than the control group ($P<0.001$). UIBC and TIBC were negatively correlated with ferritin in both sexes. Serum level of iron (OR=0.806, 95% CI (0.687-0.944), UIBC (OR=0.919, 95% CI (0.852-0.992), and TIBC (OR=0.864, 95% CI (0.787-0.95) were found to have a protective role for CAD in women ($P<0.05$, Table 3). The OR for ferritin was significant in the both sexes (OR=1.029, 95% CI (1.002-1.058) in men, OR=1.02, 95% CI (1.005-1.034) in women, $P<0.05$).

Conclusion: Low Serum level of iron, UIBC, TIBC and ferritin levels were found to have a protective role for CAD in women, but not in men. Elevated serum ferritin is independently and positively associated with CAD in men and women.

1. Introduction

Iron plays a role in several fundamental biological processes such as erythropoiesis and cell metabolism. Iron status is a modifiable feature associated with cardiovascular disease. Iron metabolism disorders, either deficiency or overload, were associated with increased cardiovascular morbidity and mortality [1, 2]. The increase of iron stores was also positively correlated with the risk factors of cardiovascular disease, such as the risk of metabolic syndrome [3], insulin resistance (IR) [4, 5] and new-onset type 2 diabetes mellitus [6]. The role of body iron indices has been reviewed in the pathogenesis of coronary artery disease (CAD) [7–11]. It has been suggested iron plays an important role in the pathogenesis of atherosclerosis by catalyzing the atherogenic modification of low density lipoprotein (LDL) [10, 12–16]. Some studies supported the iron hypothesis [4, 17–21], some found no supporting evidence, and the results of others contradicted the hypothesis [22–26].

Although iron status was implicated in cardiovascular disease, the relationship between iron states and CAD has long been a controversial topic in the literature. Because of the inconsistent results of the association between body iron stores and CAD risk, we conducted a case-control study enrolled a total of 643 CAD patients and 643 healthy controls to analyze the associations of serum iron levels with the presence of CAD, the severity of coronary artery stenosis, and 1-year rehospitalization after revascularization.

2. Subjects And Methods

Study population

The control group consisted of 643 (male/female 381/262) healthy persons without cardiovascular disease via physical examination and electrocardiogram. The controls were frequency-matched to the cases on age and sex. The CAD group consisted of 643 patients, 349 men and 294 women. All CAD patients underwent physical examination and review of their medical history. Patients were excluded if they 1) had heart failure, acute myocardial infarction, coronary bypass surgery or angioplasty, coronary spasm, or myocardial bridge; 2) had cardiac diseases such as cardiomyopathy, valvular or congenital heart disease, arrhythmia; 3) patients who had malignant tumors, acute or chronic infection, iron deficiency anemia, digestive system disease, fever, infection, connective tissue disease, autoimmune disease; 4) patients whose Blood pressure $\geq 180/110$ mmHg after taking standard antihypertensive drugs, severe hepatic or renal dysfunction; 5) A history of major surgical trauma, pregnancy, mental illness, or any other cause of active blood loss within 2 weeks. The Zhengzhou University ethics Committee approved this study (The approval number 2020-KY-172). All the investigations were performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants or from a parent or legal guardian if participants are under 16.

Methods

All subjects underwent anthropometrical evaluation with measurements of weight, height, and body mass index (BMI). Laboratory determinations: All blood samples were obtained after overnight fasting. Fasting plasma glucose (FPG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), high sensitive C-reactive protein (hs-CRP), iron metabolism biomarkers, liver function and renal function markers were measured on a Cobas 8000 Analyzer (Roche Diagnostics, Germany) using the Original Roche Reagent following the manufacturer's manual.

Plasma glucose was measured by the hexokinase method; TG, TC, LDL-C and HDL-C, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), creatinine, urea nitrogen (UREA), uric acid (UA) concentrations were evaluated by enzymatic methods. Hemoglobin A1c (HbA1c) was assayed by high-performance liquid chromatography on a Primer-Premier Hemoglobin Testing System (reagents were supplied by Primus). hs-CRP, homocysteine (Hcy), Apolipoprotein A (ApoA), Apolipoprotein B (ApoB) and serum ferritin levels were measured using an immune-turbid metric assay. The serum iron was measured with a colorimetric method; while the levels of unsaturated transferrin iron binding capacity (UIBC) was measured using a colorimetric test. Total iron binding capacity (TIBC) equaled the serum iron levels plus serum UIBC levels. Finally, estimated glomerular filtration rate (eGFR) was computed by using the chronic kidney disease epidemiology (CKD-EPI) collaboration equation [27]. Iron deficiency was defined as: ferritin <100 ng/mL or 100 – 300 ng/mL with transferrin saturation (Tfs) $<20\%$ [28]. Ferritin were categorized in low (<30 ng/mL; women CAD/Control $n=6/40$, male CAD/Control $n=6/8$), normal (30 – 200 ng/mL in women; CAD/Control $n=168/197$, and 30 – 300 ng/mL in men; CAD/Control $n=204/304$), and high (>200 ng/mL in women, CAD/Control $n=120/25$, and >300 ng/mL in men; CAD/Control $n=139/69$) ferritin levels [21, 29–31]. Selective cardioangiography (CAG) was performed in all patients using the standard Judkins technique. The localization of coronary artery disease and the rate of lumen stenosis were determined by CAG.

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation (normally distributed data). Categorical variables are expressed as the frequency and its percentage. Continuous variables were analyzed using Student's t-test in normally distributed data, and Mann-Whitney test in non-normally distributed data. Chi-squared test was

utilized for categorical variables. The association between continuous variables was assessed by Pearson correlation. The association of iron metabolism biomarkers with CAD was analyzed by logistic regression models with three progressive degrees of adjustment. Model 1 was a crude model without any confounders; model 2 was adjusted for age and cardiovascular risk factors including smoking habit, alcohol drinking habit, body mass index (BMI), hypertension, and dyslipidemia; model 3 was additionally adjusted for laboratory tests including HbA1C, ALT, AST, TC, TG, hs-CRP, eGFR. To avoid collinearity, we did not include iron and transferrin saturation into the multiple liner regression models because these two variables lied into the same causal pathway between ferritin and CAD. All the statistical analyses were executed using Statistical Package for Social Science (SPSS, version 22.0) and STATA statistical package (version 13, Texas, USA).

3. Results

Clinical characteristics

Table 1 showed the general characteristics of the study groups according to the sexual groups. There was no significant difference in age and diastolic blood pressure (DBP) between CAD group and controls. Overall, CAD patients were more likely to be smokers, alcohol drinkers, obese, hypertensive, dyslipidemia and hyperglycemia. Comparing to the controls, individuals in the male CAD group showed significantly lower plasma concentrations of Total proteins (TP), Albumin (ALB), and ApoB, Tfs serum iron levels, systolic blood pressure (SBP) and body mass index (BMI). The plasma concentrations of ferritin, UIBC, HbA1c, FBP, hs-CRP, liver function and renal function were significantly higher in the CAD group compared to the control in men ($P<0.05$) (Table 1, Figure 1). Similar difference was found in the female group (Table 1, Figure 1). Additionally, significant differences were found in the diabetes management styles between male and female patients with CAD (Table 1).

Table 1
Clinical characteristics of patients and controls

	male			female		
	Control (n=381)	CAD (n=349)	<i>P</i>	Control (n=401)	CAD (n=294)	<i>P</i>
Smoking n (%)	179 (46.98)	244(70)	<0.001	154 (38.40)	115(39.25)	0.82
Drinking n (%)	174(45.67)	128(36.68)	0.014	45 (11.22)	25 (8.53)	0.24
age(years)	53.05±10.75	58.56±10.82	0.052	49.38±12.09	61.9±10.48	0.128
BMI (kg/m2)	22.99±2.46	24.23±2.55	0.095	22.98±1.87	24.18±2.68	<0.001
SBP (mmHg)	120.32±9.28	127.92±17.07	<0.001	120.79±9.73	130.94±17.7	<0.001
DBP (mmHg)	74.84±9.04	78.76±11.14	0.426	74.38±9.02	78.45±11.8	0.457
HR(heart ratio)	70.21±5.08	77.45±10.47	<0.001	69.78±4.96	77.66±10.64	<0.001
Smoking status (%)	60(15.75)	118(33.81)	<0.001	48(11.97)	94(31.97)	<0.001
Drinker (%)	56(14.7)	101(28.94)	<0.001	45(11.22)	87(29.59)	<0.001
HbA1c (%)	5.4±0.45	6.09±1.21	<0.001	5.46±0.47	6.29±1.36	<0.001
FPG (mmol/L)	5.06±0.6	5.33±1.79	<0.001	5.04±0.7	5.58±1.88	<0.001
ALT(U/L)	24.31±17.02	42.57±125.79	0.004	17.06±9.6	40.54±225.86	0.042
AST(U/L)	22.22±11.71	32.17±38.03	<0.001	19.95±7.52	54.75±417.38	0.056
TBIL (μmol/L)	12.99±5.27	12.19±8.66	0.008	10.19±4.77	10.61±14.93	0.19
BILD (μmol/L)	5.53±1.85	5.4±4.34	0.002	4.68±1.77	5±12.31	0.156
GGT(U/L)	38.69±56.96	36.9±33.61	0.466	30.53±19.61	32.33±43.83	0.251
TP(g/L)	72.86±4.09	65.46±5.41	<0.001	74.1±3.81	66.68±5.62	<0.001
ALB(g/L)	47.32±2.82	40.94±4.02	<0.001	46.78±2.39	40.93±4.34	<0.001
ALP(U/L)	70.58±17.4	72.22±21.25	<0.001	69.72±22.4	79.45±39.34	0.001
TC (mmol/L)	4.64±0.99	3.41±0.88	0.574	4.64±0.95	3.9±1.09	0.126
TG (mmol/L)	1.74±1.07	1.49±1.03	0.052	1.29±0.7	1.58±1.25	0.046
HDL (mmol/L)	1.2±0.29	0.99±0.24	0.084	1.43±0.31	1.12±0.31	0.983
LDL (mmol/L)	2.91±0.87	1.95±0.79	0.653	2.83±0.84	2.23±0.88	0.338
APOA (g/L)	1.37±0.26	1.11±0.26	0.958	1.43±0.25	1.17±0.26	0.018
APOB (g/L)	0.86±0.27	0.84±0.36	0.028	0.84±0.21	0.94±0.42	0.002
Lpa (g/L)	0.16±0.17	0.33±1.78	0.216	0.16±0.17	0.25±0.27	<0.001
HCY (umol/L)	13.59±9.17	15.58±9.08	0.698	13.31±7.29	15.69±8.39	0.732

	male			female		
hsCRP (mg/L)	1.61±1.61	5.68±14.05	0.004	1.09±0.88	8.84±25.41	0.003
CREA (μmol/L)	80.77±12.4	87.44±81.8	0.004	63.44±12	68.59±25.08	<0.001
Urea (mmol/L)	5.1±1.23	6±3.24	<0.001	4.51±1.23	5.45±1.98	<0.001
UA (mmol/L)	365.08±89.14	319.21±100.07	0.159	270.07±62.98	286.69±95.89	<0.001
eGFR	94.17±13.18	90.39±18.27	0.001	99.36±14.61	88.3±20.48	<0.001
IRON (μmol/L)	23.35±8.46	14.14±6.4	0.001	17.8±6.59	13.55±5.91	0.283
Ferritin (ng/mL)	206.22±191.69	274.03±191.58	<0.001	92.01±79.14	233.5±178.27	<0.001
UIBC (μmol/L)	33.78±10.15	34.38±7.57	0.103	42.29±12.83	34.02±7.25	<0.001
TIBC (μmol/L)	57.14±8.13	48.52±8.9	<0.001	60.09±11.04	47.56±8.42	0.002
Tfs (transferrin saturation, %)	41.15±14.23	28.7±11.33	0.009	30.5±11.77	28.12±10.55	0.456
Iron deficiency (%)	0	65(18.6)		0	83(28.2)	<0.001
Hypertension n (%)		132(37.82)			108(36.73)	0.77
Clopidogrel n (%)		6(4.55)			5(4.63)	0.97
ACE inhibitors n (%)		10(7.58)			1(0.93)	<0.001
ARB n (%)		33(25)			29(26.85)	0.74
β-blockers n (%)		28(21.21)			20(18.52)	0.6
Calcium channel blockers n (%)		36(27.27)			30(27.78)	0.97
Diuretics n (%)		9(6.82)			10(9.26)	0.48
Diabetes n (%)		113(32.38)			85(28.91)	0.34
Diabetes management						
Lifestyle modification n (%)		15(13.27)			10(11.76)	<0.001
Oral agents only n (%)		33(29.2)			35(41.18)	<0.001
Insulin only n (%)		64(56.64)			30(35.29)	<0.001
Oral agents and insulin n (%)		1(0.88)			10(11.76)	<0.001
Family History of CAD n (%)		106(30.37)			66(22.45)	0.024

	male	female	
Dislipidemia n (%)	130(37.25)	72(24.49)	<0.001
Statins n (%)	75(57.69)	42(58.33)	0.93
Rehospitalized ratio	114(32.66)	77(26.19)	0.072

Correlation between ferritin and other metabolic variables in CAD patients

We analyzed the correlation between ferritin and other metabolic variables in CAD by Pearson correlation. We totally tested the correlation between serum ferritin and 17 biomarkers, i.e. blood lipids (LDL-C, HDL-C, TC, TG, ApoA, ApoB and Lp(a)), blood glucose (FPG, HbA1c), blood pressure (systolic blood pressure, SBP and diastolic blood pressure, DBP), eGFR, proinflammatory measures (hs-CRP), adiposity measure (BMI) and other iron metabolism markers. TC, TG, LDL-C and Tfs were positively correlated with ferritin in men, HDL-C, ApoA, and eGFR were negatively correlated with ferritin in women, while BMI was inversely associated with ferritin in women (Table 2). UIBC and TIBC were negatively correlated with ferritin in both sexes. Interestingly, HbA1C level was positively correlated with ferritin in men, while inversely associated with ferritin in women.

Table 2
Correlation between ferritin and other metabolic variables in CAD patients

	male(n=349)		female(n=294)	
variables	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
eGFR	0.023	0.706	-0.267	<0.001
Blood lipids				
TC (mmol/L)	0.131	0.003	-0.075	0.154
TG (mmol/L)	0.198	<0.001	0.085	0.105
HDL (mmol/L)	-0.033	0.450	-0.196	<0.001
LDL (mmol/L)	0.107	0.014	-0.059	0.263
APOA(g/L)	-0.066	0.254	-0.168	0.008
APOB (g/L)	0.053	0.360	0.077	0.222
Lpa (g/L)	-0.032	0.584	0.078	0.195
Blood glucose				
HbA1C (%)	-0.085	0.041	0.188	<0.001
Blood pressure				
SBP (mmHg)	0.069	0.095	0.069	0.167
DBP (mmHg)	0.029	0.486	0.059	0.234
Inflammation				
hsCRP(mg/L)	0.049	0.541	-0.089	0.341
Adiposity				
BMI (m/kg2)	0.041	0.324	0.115	0.02
Iron metabolic markers				
IRON (μmol/L)	.081	0.051	-0.216	<0.001
UIBC (μmol/L)	-0.262	<0.001	-0.295	<0.001
TIBC (μmol/L)	-0.168	<0.001	-0.393	<0.001
Tfs (transferrin saturation, %)	0.128	0.002	-0.096	0.054
Bold values indicate significant results with $P < 0.05$.				
eGFR: estimated glomerular filtration rate, BMI: body mass index, HbA1c: glycosylated haemoglobin, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol.				
lipoprotein cholesterol, TG triglycerides, SBP systolic blood pressure, DBP: diastolic blood pressure, hs-CRP: high sensitive C-reactive protein.				
Pearson correlation was performed with ferritin and other metabolic variables in CAD patients. Continuous variables with skewed distributions (ferritin, TG,hs-CRP) were log transformed for analysis.				

Logistic regression analysis of iron metabolic markers and the risk of CAD and 1-year rehospitalization

To illustrate the continuous relationships between parameters of iron metabolism and the risk of CAD, we assessed the concentration-risk relationship between serum UIBC, ferritin levels and CAD risk by multivariate random-effects meta-regression based on the restricted cubic spline model with four knots (Figure 2). Visual inspection revealed J-shaped relationships between UIBC, ferritin and the ORs for the risk of CAD. The association between ferritin and high CAD was further explored by categorizing ferritin levels into low, normal and high groups, and using the normal ferritin level as the reference.

From an unadjusted multivariable logistic regression analysis, each unit increase in ferritin was associated with a 1.003-fold (95% confidence interval (CI), 1.002-1.004) and 1.01-fold (95% CI, 1.000-1.01) increased Odds Ratio (OR) of CAD in male and female groups respectively (Table 3). Serum iron level and Tfs were found to have decreased OR for CAD in both sexes (iron OR=0.83, 95% CI 0.803-0.858 in men, OR=0.894, 95% CI 0.860-0.929 in women, Tfs OR=0.896, 95% CI 0.867-0.925 in men, OR=0.977, 95% CI 0.957-0.997 in women, $P<0.05$, Table 3). Patients with low serum level of UIBC, TIBC, and Iron deficiency had significantly lower risks for CAD in women (OR=0.214, 95% CI (0.145-0.315), $P<0.001$, Table 3).

Table 3
Associations of iron metabolic markers and CAD

Models	male (Control/CAD 381/349)		Female (Control/CAD 262/294)	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Model 1				
Iron (μmol/L)	0.83(0.803-0.858)	<0.001	0.894(0.860-0.929)	<0.001
Ferritin (ng/mL)	1.003(1.002-1.004)	<0.001	1.01(1-1.01)	0.017
UIBC (μmol/L)	1.008(0.989-1.027)	0.417	0.88(0.79-0.98)	0.018
TIBC (μmol/L)	0.961(0.825-1.12)	0.613	0.89(0.86-0.92)	<0.001
Normal ferritin	1 (Reference)			
low ferritin	0.466(0.306-0.709)	<0.001	0.2(0.1-0.4)	<0.001
high ferritin	0.371(0.102-1.353)	0.133	0.12(0.03-0.42)	0.001
Tfs (transferrin saturation, %)	0.89(0.869-0.911)	<0.001	0.977(0.957-0.997)	0.022
Iron deficiency	1.203(0.77-1.881)	0.417	0.214(0.145-0.315)	<0.001
Model 2				
Iron (μmol/L)	0.841(0.805-0.878)	<0.001	0.888(0.847-0.931)	<0.001
Ferritin (ng/mL)	1.005(1.003-1.007)	<0.001	1.010(1.007-1.013)	<0.001
normal ferritin	1 (Reference)		1 (Reference)	
low ferritin	0.297(0.175-0.504)	<0.001	0.265(0.111-0.635)	0.003
high ferritin	0.168(0.032-1.072)	0.092	0.269(0.048-1.503)	0.135
Tfs (transferrin saturation, %)	0.929(0.909-0.949)	<0.001	0.862(0.82-0.905)	<0.001
UIBC (μmol/L)	/	/	0.824(0.775-0.876)	<0.001
TIBC (μmol/L)	/	/	0.873(0.840-0.907)	<0.001
Iron deficiency	/	/	8.905(0.635-124.873)	0.105
Model 3				
Iron (μmol/L)	0.889(0.626-1.264)	0.513	0.806(0.687-0.944)	0.008
Ferritin (ng/mL)	1.029(1.002-1.058)	0.037	1.02(1.005-1.034)	0.008
normal ferritin	1 (Reference)		1 (Reference)	
low ferritin	39.138(0.941-162.564)	0.054	0.087(0.01-0.724)	0.024
high ferritin	/	/	0.072(0.005-0.999)	0.05
Tfs (transferrin saturation, %)	0.87(0.695-1.089)	0.223	0.977(0.896-1.066)	0.599
UIBC (μmol/L)	/	/	0.919(0.852-0.992)	0.031

Models	male (Control/CAD 381/349)		Female (Control/CAD 262/294)	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
TIBC (μmol/L)	/	/	0.864(0.787-0.95)	0.002
Model 1 is crude model without any confounders				
Model 2 was adjusted for age, BMI, SBP, DBP, Heart ratio, smoking habit, alcohol drinking habit, hypertension, diabetes status, dyslipidemia, medicine and family history of CAD.				
Model 3 additionally adjusted for HbA1C, ALT, AST, TCHO, TG, hs-CRP, eGFR.				

When further adjusted for age, BMI, SBP, DBP, HR, smoking habit, alcohol drinking habit, hypertension, diabetes status, dyslipidemia, medicine and family history of CAD (model 2), the effect modification by serum iron, ferritin levels and Tfs on the CAD risk remained similar significant in both sexes. Additional adjustment for laboratory tests including HbA1C, ALT, AST, TCHO, TG, hs-CRP, eGFR (model 3) there was no significant direct correlation for serum iron and Tfs on the CAD risk in men ($P>0.05$). Serum level of iron (OR=0.806, 95% CI (0.687-0.944)), UIBC OR=0.919, 95% CI (0.852-0.992), and TIBC (OR=0.864, 95% CI (0.787-0.95)) were found to have a protective role for CAD in women ($P<0.05$, Table 3). The OR for ferritin was significant in the both sexes (OR=1.029, 95% CI (1.002-1.058) in men, OR=1.02, 95% CI (1.005-1.034) in women, $P<0.05$, Table 3). When analyzed with the normal ferritin group as the reference, the low level of serum ferritin showed a decreased OR for CAD in women (OR=0.087, 95% CI (0.01-0.724) $P<0.05$, Table 3).

Regrettably, there was no statistical significance between the correlation of iron metabolism markers and 1-year rehospitalization after calibrating for confounders (model 3, $P>0.05$, Table 4).

Table 4

Associations of iron metabolic markers and 1-year rehospitalization after coronary revascularization

Models	male (Control/CAD 381/349)		female (Control/CAD 262/294)	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Model 1				
Iron (μmol/L)	0.944(0.917-0.973)	<0.001	0.8(0.64-1.01)	0.059
Ferritin (ng/mL)	1.001(1-1.003)	0.026	1(0.996-1.01)	0.276
UIBC (μmol/L)	0.997(0.973-1.022)	0.839	1.04(0.96-1.13)	0.369
TIBC (μmol/L)	0.961(0.939-0.983)	0.001	0.96(0.94-0.99)	0.01
Normal ferritin	1 (Reference)		1 (Reference)	
low ferritin	0.677(0.429-1.071)	0.095	0.45(0.19-1.06)	0.068
high ferritin	0.75(0.152-3.695)	0.724	0.05(0-0.59)	0.018
Tfs (transferrin saturation, %)	0.977(0.961-0.994)	0.008	0.977(0.961-0.994)	0.155
Iron deficiency	0.882(0.509-1.53)	0.665	0.882(0.509-1.53)	0.67
Model 2				
Iron (μmol/L)	0.95(0.92-0.981)	0.002	0.96(0.91-1)	0.052
Ferritin (ng/mL)	1.001(1-1.003)	0.027	1.001(0.999-1.002)	0.526
normal ferritin	1 (Reference)		1 (Reference)	
low ferritin	/	/	0.81(0.46-1.43)	0.462
high ferritin	/	/	0.2(0.02-1.89)	0.161
Tfs (transferrin saturation, %)	0.98(0.963-0.998)	0.032	/	/
UIBC (μmol/L)	/	/	0.99(0.96-1.03)	0.662
TIBC (μmol/L)	0.967(0.943-0.991)	0.007	0.971(0.944-0.999)	0.043
Iron deficiency	/	/	0.982(0.959-1.006)	0.141
Model 3				
Iron (μmol/L)	0.972(0.916-1.031)	0.345	1.032(0.924-1.154)	0.574
Ferritin (ng/mL)	1.001(0.998-1.003)	0.576	/	/
Tfs (transferrin saturation, %)	0.979(0.945-1.013)	0.226	/	/
TIBC (μmol/L)	1.009(0.959-1.062)	0.733	1.015(0.947-1.088)	0.674
Model 1 is crude model without any confounders				
Model 2 was adjusted for age, BMI, SBP, DBP, Heart ratio, smoking habit, alcohol drinking habit, hypertension, diabetes status, dyslipidemia, medicine and family history of CAD.				

Models	male (Control/CAD 381/349)		female(Control/CAD 262/294)	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Model 3 additionally adjusted for HbA1C, ALT, AST, TC, TG, hs-CRP, eGFR.				

Association of body iron status with the severity of CAD

The severity of CAD was quantified the modified Gensini scores based on the number and the extent of lesions in coronary arteries [32]. The detail calculation of modified Gensini scores was described in our previous study[33]. Serum iron status markers exhibited no significant association with the severity of CAD (Supplement Table 1).

4. Discussion

There are limited data concerning serum iron and iron saturation in CAD patients and the results are also inconsistent. This study was conducted to assess the association between serum iron metabolism markers and CAD. For physiological reasons, the reference interval for iron metabolism markers in women are different from men, we analyzed the association of iron status and CAD stratified by gender. In a cohort of 643 CAD patients and 643 controls, iron imbalance, as characterized by either high serum ferritin or low iron levels, was associated with an increased risk of CAD.

Overall, our findings suggested that low Serum level of iron, UIBC, TIBC and ferritin levels showed a protective role for CAD in women, but not in men. Increased serum ferritin was independently associated with CAD in men and women. Our data add to previous reports that showed a protective role of low iron level on the risk of CAD. Sullivan proposed that reduced iron stores can protect against ischemic heart disease and for the first time to explain the sex difference in CAD risk [34]. Salonen, J.T. et al. observed that a higher level of iron is a risk factor for myocardial infarction in Finnish men [35]. Later, it has been reported that reducing iron stores through phlebotomy could decelerate the progression of atherosclerotic plaque [12, 22]. However, we did not find iron deficiency was associated with increased risk for CAD, which was inconsistent with some previous report. Iron deficiency was associated with increased risk for CAD and had detrimental effects in patients with CAD [1, 26]. The patients' status and comorbidities might explain the inconsistency. In our study, we enrolled low percentage (less than 30%) of iron deficiency in the patient group, and we exclude the CAD patients with clinical anemia. However, there was a high prevalence of iron deficiency in acute coronary syndrome and its association with poor outcome [36]. Moreover, the definition of iron deficiency to be applied in heart disease remains controversial.

Iron is a trace element that exists in serum at low concentration of mg/dL. Iron values exhibit diurnal variation depending on dietary iron intake or patient condition [37]. The iron measurements may be affected by recent food ingestion. Various previous studies have evaluated serum ferritin instead of serum iron level. Ferritin is an iron-binding molecule that stores iron in a biologically available form, which is essential to iron homeostasis [38]. Moreover, serum ferritin is a well-known acute-phase reactant. Nevertheless, there are inconsistent results concerning the relation of serum ferritin with CAD. A batch of studies have shown a similar positive correlation between ferritin and the risk of CAD [7, 17, 19, 20, 23, 24, 39–41]. Elevated serum ferritin was independently significantly associated with carotid atherosclerosis in women (Xu et al., 2017). Consistent with previous positive results, in our study, we found serum ferritin was independently significantly associated with CAD in both sexes. On the other hand, some other studies demonstrated that the higher risk of CAD is not related to the serum ferritin

levels [24, 42]. These studies implied that ferritin might be an inflammatory marker for atherosclerosis. It is hard to tell whether high ferritin levels reflect inflammation caused by hyperglycemia or indicate iron overload, which can also lead to inflammation.

Iron is an essential mineral, which participates in different functions of the organism under physiological conditions. Numerous biological processes, such as oxygen and lipid metabolism, protein production, cellular respiration, and DNA synthesis, require the presence of iron [43]. Maintaining iron metabolism is important for cardiovascular health as its high energy consumption and high mitochondrial activity [44]. Intravenous iron administration in acute myocardial infarction (MI) exerted beneficial effects in MI patients [45]. However, excessive iron accumulation accelerates the formation of atherosclerosis through several putative mechanisms. Firstly, iron catalyzes Fenton reaction to produce reactive oxygen species (ROS) which promote LDL peroxidation and induce endothelial dysfunction by reducing the bioavailability of nitric oxide [46]. Secondly, excess iron may make vascular endothelium vulnerable to other pathogenic factors. Ultimately, atherosclerosis is accelerated as a result of increased platelet activity and leucocyte adhesion [47]. Thirdly, ROS increased the expression of LOX-1 receptor on endothelial cells, resulting in mitochondrial DNA damage and autophagy activation [48]. Fourthly, accumulated iron in adipocytes leads to adipocyte IR by increasing lipolysis and by decreasing insulin-stimulated glucose transport [2]. Adipocyte IR accelerates atherosclerosis [49].

5. Conclusion

There are limited data concerning serum iron and iron saturation in CAD patients and the results are also inconsistent. The discrepancies among the studies may be partly attributable to the differences in race, dietary habits, sample size and confounding factors. Low Serum level of iron, UIBC, TIBC and ferritin levels were found to have a protective role for CAD in women, but not in men. Increased serum ferritin is independently associated with CAD in men and women. The main limitation of the study is its cross-sectional design; therefore, a causal relationship could not be established. In addition, the measurements of hepcidin, a well-known regulator of body iron fluxes, were not available. However, we evaluated transferrin saturation which is an important determinant of hepcidin release. Moreover, the daily iron intake needs to be estimated in the future study. Finally, there is a pragmatic need to identify circulating iron biomarkers reliably characterizing iron status within tissues.

6. Abbreviations

UIBC: unsaturated transferrin iron binding capacity; TIBC: Total transferrin iron binding capacity; TC: total cholesterol; TG: Triglyceride; CAG: cardioangiography; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hs-CRP: High sensitivity C reactive protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ -GT: γ -glutamyl transferase; eGFR: Estimated glomerular filtration rate; BP: Blood pressure; Tfs: transferrin saturation; IR: insulin resistance; TC: total cholesterol; UREA: urea nitrogen; UA: uric acid; HbA1c: Hemoglobin A1c; Hcy: homocysteine; ApoA: Apolipoprotein A; ApoB: Apolipoprotein B; TP: Total proteins; ALB: Albumin.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

Each author is expected to have made substantial contributions. Shuren Guo performed the experiments, analyzed the data, prepared figures and tables, and reviewed drafts of the paper. Xiaohuan Mao performed the data collection and analysis, Xiaohua Li, Huan Ouyang and Yuhua Gao performed the laboratory tests and clinical data collection. Liang Ming have drafted the work and substantively revised it. All authors have approved the submitted version and agreed to be accountable for all aspects of work ensuring integrity and accuracy. All authors read and approved the final manuscript.

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Figures

Figure 1

The serum levels of iron metabolism markers in CAD and Control groups stratified by gender.

Figure 2

Concentration-risk relationship between serum UIBC (A), ferritin levels (B) and risk of CAD. Solid lines show cubic spline interpolation of ORs versus quartile medians, which were all adjusted (model3) for age, sex, BMI, SBP, DBP, HR, smoking habit, alcohol drinking habit, hypertension, diabetes status, dyslipidemia, medicine and family history of CAD, HbA1C, ALT, AST, TC, TG, hs-CRP and eGFR.

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