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Increased serum ferritin levels are independently associated with carotid atherosclerosis in women

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Abstract

Previous studies have supported the theory that there is a positive association between ferritin and carotid atherosclerosis in Western people. Diet plays an important role in determining serum ferritin concentration. Asian dietary patterns are different from Western dietary patterns, implying that there may be a difference in the association of ferritin with carotid atherosclerosis between Asian and Western people. However, few studies focus on the association between ferritin and carotid atherosclerosis among Asians. The aim of this study was to investigate how serum ferritin levels are associated with carotid atherosclerosis in an Asian adult population. A cross-sectional assessment was performed in 8302 adults in Tianjin, China. Carotid intima-media thickness (IMT) and plaques were assessed using ultrasonography, and serum ferritin was measured using the protein chip-chemiluminescence method. Multiple logistic regression analysis was used to examine the association between quartiles of serum ferritin concentration and carotid atherosclerosis. In the present study, the overall prevalence of IMT and carotid plaques in participants is 29·2 and 22·7 %, respectively. In women, after adjustments for potentially confounding factors, the OR of IMT and carotid plaques by increasing serum ferritin quartiles were 1·00, 1·39 (95 % CI 0·98–1·99), 1·39 (95 % CI 0·99–1·97), 1·81 (95 % CI 1·30–2·55) ($P_{\text{for trend}} < 0·001$) and 1·00, 1·24 (95 % CI 0·89–1·73), 1·18 (95 % CI 0·85–1·65), 1·59 (95 % CI 1·15–2·20) ($P_{\text{for trend}} < 0·01$), respectively. However, no association was found between serum ferritin and carotid atherosclerosis in men. The study demonstrated that increased serum ferritin levels are independently associated with IMT and carotid plaques in Asian women but not in Asian men.

Key words: Ferritin: Carotid atherosclerosis: Carotid intima-media thickness: Carotid plaques: Asian population

CVD is considered a global public health challenge and it is a major cause of morbidity and mortality in the general population[1]. Atherosclerosis, a chronic inflammatory disease of the artery wall, is the underlying cause of CVD[2]. Atherosclerosis is highly prevalent in older people[3]. According to a multi-ethnic study of carotid atherosclerosis in America (mean age 69·1 years), about 18 % of individuals have atherosclerotic lesions, as determined by ultrasonography[4]. In China, the prevalence of atherosclerotic lesions is up to 12·4 % in middle-aged adults (mean age 59·3 years)[5].

Oxidative stress is the unifying mechanism for many common risk factors such as endothelial dysfunction and inflammatory response, which are believed to be involved in atherosclerosis[6–8]. Oxidised LDL, modified by reactive oxygen species (ROS), plays a crucial role in the development of endothelial dysfunction by decreasing the bioavailability of nitric oxide[9–11]. Moreover, excessive production of ROS increases the expression of lectin-like oxidised LDL receptor-1 (*LOX-1*)[12]. This, in turn, increases endothelial inflammation by inducing mitochondrial DNA damage and by activating autophagy[12]. On the other hand, abnormal glucose metabolism is also associated with atherosclerosis[13]. Insulin resistance (IR) promotes a proinflammatory and pro-oxidant state that accelerates atherosclerosis formation[13].

Fe is one of essential microelements and plays an important role in blood O_2 transport[14]. The measurement of serum ferritin concentration is considered to be the best non-invasive indicator of body Fe stores[15]. Body Fe acts as a catalyst that generates ROS via the Haber–Weiss and Fenton reactions[16,17]. Moreover, excessive Fe exhibits proatherogenic properties due

Abbreviations: FBG, fasting blood glucose; IMT, intima-media thickness; ROS, reactive oxygen species.

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to its ability to generate ROS[18,19]. In addition, systemic Fe overload has been shown to be associated with adipocyte IR[20]. Given this, we hypothesise that excessive Fe accumulation promotes the formation and progression of atherosclerosis.

Several small-scale cross-sectional studies and a cohort study (n 124–2443) assessed the association between serum ferritin and carotid atherosclerosis in Western people[21–29]. On the other hand, dietary factors (especially animal foods) play an important role in body Fe status[30]. The Western dietary pattern is characterised by a high intake of red meat[31]. In contrast, the dietary patterns in Asia are characterised by a high consumption of vegetables and cereals and a low consumption of red meat[32]. Besides, the haemochromatosis (*HFE*) gene, which is one of the major regulators of Fe homoeostasis, was found to have differing genetic variants in European and Asian populations[33]. These lines of evidence implied that there may be a difference in the association of serum ferritin with carotid atherosclerosis between Asian and Western people. However, studies on a general Asian population are scarce.

The aim of this large-scale cross-sectional study was to investigate associations between serum ferritin and carotid atherosclerosis using carotid intima-media thickness (IMT) and plaques in an Asian adult population.

Methods

Study population

The observational data were derived from the Tianjin Chronic Low-grade Systemic Inflammation and Health (TCLSIHealth) Cohort Study, a large prospective dynamic cohort study focused on the association between chronic low-grade systemic inflammation and health status. More details regarding the sample population have been described in another study[34]. Study participants were employed in a wide variety of occupations or were retired and living in residential communities. Thus, the sample population in this study could represent the general adult population in Tianjin. This study was approved by the institutional review board at Tianjin Medical University. All participants provided written informed consent before participating.

The data from TCLSIHealth from 2013 to 2015 were analysed in this study. During this survey period, there were 10 027 participants who had undergone at least one health examination including carotid ultrasonography. The study excluded participants who had not undergone ferritin testing (n 247), or had a history of anaemia (n 458), CVD (n 850) or cancer (n 170). After these exclusions, a total of 8302 participants were included in the final analysis, including 4964 men (mean age 51·2 (SD 10·1) years) and 3338 women (mean age 52·2 (SD 10·8) years).

Carotid ultrasonography

Trained sonographers performed the carotid ultrasonography using iU Elite (Royal Philips) equipped with a L9-3 transducer to measure the IMT. All participants were asked to stay in the supine position and were examined with the head turned 45° to the contralateral side of the artery. The protocol for measuring IMT involved scanning the common carotid artery (CCA) far wall (defined as the 10-mm section at a distance of 1 cm from the bifurcation) and carotid bifurcation on both left and right carotid arteries. IMT was measured as the distance from the edge of the first echogenic line to the edge of the second echogenic line. IMT was defined as a CCA IMT ≥1·0 mm or a carotid bifurcation IMT ≥1·2 mm. The procedure for detecting plaques involved scanning the near and far walls of the CCA, the carotid bifurcation, the external carotid artery and the internal carotid artery. Carotid plaques were defined as an IMT ≥1·5 mm, and plaque prevalence was defined as the presence of ≥1 plaque. Each measurement was repeated three times. The intrameasure and intermeasure CV were <2·9 %.

Serum ferritin tests

Blood samples were obtained after a 12-h overnight fast by venepuncture of the cubital vein early in the morning. The serum ferritin concentrations were determined by the protein chip-chemiluminescence method using the Quantitative Kit for Tumor Markers (Huzhou Shukang Biological Technology) with a measurement range of 5–600 ng/ml. The intra- and inter-assay CV were <15 %.

Laboratory analyses

For the analysis of fasting blood glucose (FBG) and lipids, blood samples were collected in siliconised vacuum plastic tubes. Levels of FBG were measured using the glucose oxidase method. The lipids, including total cholesterol (TC) and TAG, were measured using enzymatic methods. LDL-cholesterol was measured using the polyvinyl sulphuric acid precipitation method, and HDL-cholesterol was measured using the chemical precipitation method. As described above, all tests were analysed on the Roche Cobas 8000 modular analyzer (Roche).

General examination

Anthropometric parameters (height and weight) were recorded according to a standard protocol, and BMI was calculated as weight (kg)/height (m^2). Waist circumference was measured at the umbilical level with subjects standing and breathing normally. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the upper right arm using an automatic device (KD598; Andon). Participants were asked to rest in a seated position for at least 5 min before measuring; the mean of two measurements was taken as the final blood pressure value. Sociodemographic variables including age and sex, disease history, family history as well as current medication, menopausal, smoking (defined as 'smoker', 'ex-smoker' and 'non-smoker')and drinking status (defined as 'everyday', 'sometime', 'ex-drinker' and 'non-drinker') were noted from 'yes' or 'no' responses to relevant questions on a questionnaire.

Definition

Anaemia was defined as having Hb concentration <130 g/l in men and <120 g/l in women or as having serum ferritin

concentration <12·5 ng/ml in men and <5·5 ng/ml in women. Hypertension was defined as having an average SBP ≥140 mmHg, an average DBP ≥90 mmHg or as the use of antihypertension medications. Hyperlipidaemia was defined as having TC ≥5·17 mmol/l, TAG ≥1·7 mmol/l, LDL-cholesterol ≥3·37 mmol/l or as the use of antihyperlipidaemic medications. Diabetes was defined as having FBG levels ≥7·0 mmol/l, oral glucose tolerance test values ≥11·1 mmol/l, HbA1c ≥48 mmol/mol (6·5 %) or a history of diabetes, which is in accordance with the latest recommendations from the American Diabetes Association[35]. The metabolic syndrome was defined according to the criteria of the American Heart Association scientific statements of 2009[36].

Statistical analysis

All statistical analyses in the present study were performed using the Statistical Analysis System 9.3 edition for Windows (SAS Institute). Continuous variables are presented as the geometric means and 95 % CI after logarithmic transforming, and categorical variables are shown as percentages. For analysis, the IMT and carotid plaques were used as dependent variables, and the serum ferritin levels were used as independent variables. Participants were divided into four categories according to quartiles of serum ferritin concentrations. For characteristics analysis, differences among serum ferritin categories were examined using ANCOVA for continuous variables or using multiple logistic regression analysis for categorical variables. Associations between serum ferritin levels and carotid atherosclerosis were examined using logistic regression in three different models; OR and 95 % CI were calculated. Analysis was performed without any adjustment in model 1; the analysis was adjusted for age and BMI in model 2; model 3 additionally adjusted for waist circumference, smoking status, drinking status, hypertension, hyperlipidaemia, diabetes, the metabolic syndrome as well as for family history of CVD, hypertension, hyperlipidaemia and diabetes. The median value of each serum ferritin quartile was used to calculate the P values for linear trends. Interactions between ferritin concentrations and confounders of carotid atherosclerosis were tested by addition of cross-product terms to the regression model. The analysis applied a two-tailed significance test and considered $P<0·05$ as an indication of statistical significance.

Results

In the present study, the overall prevalence of IMT and carotid plaques in participants were 29·2 and 22·7 %, respectively. Characteristics of participants according to serum ferritin categories are shown in Table 1. In men, compared with those in the lowest quartiles, participants in the highest serum ferritin quartiles tended to be younger and have higher BMI, waist circumference, TC, TAG, FBG and LDL levels, but lower HDL levels ($P_{\text{for trend}}\leq0·03$). Besides, more participants in the highest quartiles had the metabolic syndrome, hyperlipidaemia and diabetes, and consumed more alcohol ('non-drinker', 'sometime') ($P_{\text{for trend}}<0·01$). In women, compared with participants in the lowest serum ferritin quartiles, those in the highest quartiles were older and more likely to have a higher BMI, waist circumference, TC, LDL, TAG, SBP, DBP and FBG, but lower HDL levels ($P_{\text{for trend}}<0·0001$). A higher proportion of women in the highest quartiles had the metabolic syndrome, hypertension, hyperlipidaemia, diabetes and a family history of hypertension and hyperlipidaemia; in addition, they were more likely to be current smokers and to drink less ('non-drinker', 'sometime') ($P_{\text{for trend}}\leq0·05$). Except for these results, no significant differences were observed among participants in the four serum ferritin categories.

The crude and adjusted associations between serum ferritin and carotid IMT are presented in Table 2. In the women from the study group, serum ferritin levels were positively associated with carotid IMT in all models. After final multiple adjustment, the OR of IMT for serum ferritin across the quartiles were 1·00, 1·39 (95 % CI 0·98, 1·99), 1·39 (95 % CI 0·99, 1·97) and 1·81 (95 % CI 1·30, 2·55) ($P_{\text{for trend}}<0·001$). In men, after the final adjustment, the OR of IMT across serum ferritin quartiles were as follows: 1·00, 0·98 (95 % CI 0·81, 1·19), 1·15 (95 % CI 0·95, 1·39) and 0·91 (95 % CI 0·75, 1·10) ($P_{\text{for trend}}=0·53$). Similar results were observed in multiple logistic regression analysis for associations between serum ferritin levels and carotid plaques (Table 3). In model 3, the OR of carotid plaques for increasing quartiles of serum ferritin in women and men were 1·00, 1·24 (95 % CI 0·89, 1·73), 1·18 (95 % CI 0·85, 1·65) and 1·59 (95 % CI 1·15, 2·20) ($P_{\text{for trend}}<0·01$); 1·00, 0·99 (95 % CI 0·82, 1·21), 1·12 (95 % CI 0·92, 1·36) and 0·85 (95 % CI 0·69, 1·04) ($P_{\text{for trend}}=0·17$), respectively.

In this study, the proportion of postmenopausal women was 62·4 %. Because menstruation significantly influences Fe stores[37], stratification analyses were performed by menopausal status. However, similar associations were observed when premenopausal and postmenopausal women were analysed separately ($P_{\text{for interactions}}\geq0·24$).

Discussion

This cross-sectional study was conducted to assess the association between serum ferritin concentration and carotid atherosclerosis. The results suggested that increased serum ferritin is independently associated with carotid IMT and plaques in women, but not in men. To our knowledge, this is the first large-scale study concerning the topic of serum ferritin concentration and carotid atherosclerosis in an Asian general population.

Several cross-sectional studies have investigated the association between serum ferritin and carotid atherosclerosis in the Western general population. The results of these studies are inconsistent. Four of the studies performed analyses in men or women[23–26], with one study identifying a positive association between serum ferritin and carotid plaques in men[25], another study finding a positive association between serum ferritin and carotid plaques only in women[26], and the two remaining studies finding no association between serum ferritin and carotid atherosclerosis[23,24]. In addition, four studies have combined men and women together in the statistical analyses[22,27–29]. Two studies focusing on carotid plaques

Table 1. Participant characteristics by categories of serum ferritin concentration (Geometric least square means and 95 % confidence intervals)

	Quartiles of serum ferritin concentration (ng/ml)								
	Level 1		Level 2		Level 3		Level 4		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	$P_{for\ trend}$*
Men									
No. of subjects		1242		1241		1240		1241	–
Age (years)	51·70	51·12, 52·29	50·27	49·71, 50·84	49·42	48·87, 49·99	49·20	48·65, 49·76	<0·0001
BMI (kg/m^2)	25·61	25·44, 25·78	25·78	25·61, 25·96	26·44	26·26, 26·62	26·67	26·49, 26·85	<0·0001
Waist circumference (cm)	89·90	89·42, 90·37	90·47	89·99, 90·95	92·06	91·57, 92·54	92·59	92·10, 93·08	<0·0001
TC	4·95	4·90, 5·00	4·95	4·90, 5·00	5·09	5·04, 5·14	5·10	5·05, 5·15	<0·0001
LDL-cholesterol	2·92	2·87, 2·96	2·93	2·89, 2·98	3·01	2·96, 3·06	2·94	2·89, 2·99	0·03
HDL-cholesterol	1·24	1·22, 1·26	1·21	1·19, 1·23	1·17	1·15, 1·19	1·14	1·12, 1·15	<0·0001
TAG	1·46	1·42, 1·50	1·52	1·47, 1·56	1·72	1·67, 1·77	2·00	1·94, 2·06	<0·0001
SBP	126·57	125·67, 127·48	125·80	124·90, 126·70	126·25	125·34, 127·15	125·97	125·07, 126·88	0·66
DBP	81·63	81·00, 82·27	81·56	80·93, 82·20	81·96	81·32, 82·60	82·39	81·76, 83·04	0·25
FBG	5·24	5·19, 5·30	5·25	5·20, 5·31	5·38	5·32, 5·44	5·54	5·48, 5·61	<0·0001
MetS (%)		39·29		42·06		49·15		56·49	<0·0001
Hypertension (%)		46·94		47·06		47·02		47·95	0·61
Hyperlipidaemia (%)		61·43		60·84		68·06		75·83	<0·0001
Diabetes (%)		8·29		6·45		8·95		12·41	<0·0001
Smoking status (%)									
Smoker		45·56		45·70		46·59		48·10	0·19
Ex-smoker		9·88		8·52		9·85		9·12	0·84
Non-smoker		44·56		45·79		43·55		42·78	0·23
Drinker (%)									
Every day		7·56		8·41		7·67		10·11	0·07
Sometimes		66·25		68·50		70·19		71·69	<0·01
Ex-drinker		5·49		4·64		4·86		3·93	0·16
Non-drinker		20·70		18·45		17·28		14·27	<0·001
Family history of diseases (%)									
CVD		43·08		41·02		39·76		39·24	0·051
Hypertension		56·12		54·71		54·84		53·83	0·30
Hyperlipidaemia		8·70		9·27		10·48		8·30	0·82
Diabetes		36·39		38·11		37·98		36·66	0·97
Women									
No. of subjects		835		834		834		835	–
Age (years)	44·59	43·99, 45·19	48·59	47·94, 49·25	53·95	53·23, 54·68	57·78	57·00, 58·56	<0·0001
BMI (kg/m^2)	23·31	23·10, 23·53	24·02	23·80, 24·24	24·45	24·23, 24·68	25·16	24·93, 25·39	<0·0001
Waist circumference (cm)	77·55	76·96, 78·15	79·77	79·17, 80·38	81·85	81·23, 82·48	84·86	84·22, 85·51	<0·0001
TC	4·79	4·73, 4·85	5·05	4·99, 5·12	5·23	5·16, 5·30	5·37	5·30, 5·44	<0·0001
LDL-cholesterol	2·70	2·65, 2·76	2·92	2·87, 2·98	3·05	2·99, 3·11	3·19	3·13, 3·25	<0·0001
HDL-cholesterol	1·50	1·48, 1·53	1·48	1·45, 1·50	1·49	1·46, 1·51	1·40	1·37, 1·42	<0·0001
TAG	1·02	0·98, 1·05	1·12	1·09, 1·16	1·21	1·17, 1·25	1·39	1·35, 1·44	<0·0001
SBP	116·58	115·39, 117·78	120·42	119·19, 121·66	125·44	124·16, 126·74	130·22	128·89, 131·56	<0·0001
DBP	73·18	72·47, 73·89	75·04	74·32, 75·78	76·52	75·78, 77·27	77·99	77·23, 78·75	<0·0001
FBG	4·91	4·85, 4·96	5·02	4·97, 5·08	5·16	5·11, 5·22	5·42	5·36, 5·48	<0·0001
MetS (%)		14·85		23·53		30·34		46·47	<0·0001
Hypertension (%)		22·63		29·26		39·57		48·86	<0·0001
Hyperlipidaemia (%)		41·08		55·28		60·55		69·22	<0·0001
Diabetes (%)		1·68		3·00		4·92		9·22	<0·0001
Smoking status (%)									
Smoker		2·62		4·23		4·46		6·61	<0·001
Ex-smoker		0·55		0·68		0·56		0·28	0·38
Non-smoker		96·83		95·09		94·98		93·11	<0·01
Drinker (%)									
Every day		0·51		0·49		0·36		0·55	0·96
Sometimes		34·51		29·59		28·14		26·14	<0·01
Ex-drinker		4·71		4·39		3·23		4·20	0·54
Non-drinker		60·27		65·53		68·28		69·10	<0·01
Family history of diseases (%)									
CVD		38·20		43·65		38·61		36·65	0·12
Hypertension		54·49		56·24		55·04		50·42	0·047
Hyperlipidaemia		8·14		8·75		7·43		5·27	<0·01
Diabetes		35·09		38·25		36·45		33·77	0·31

TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; MetS, metabolic syndrome.
* ANOVA or logistic regression analysis.

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Table 2. Adjusted associations between categories of serum ferritin concentration and carotid intima-media thickness (IMT) (Adjusted odds ratios and 95 % confidence intervals)

	Quartiles of serum ferritin concentration (ng/ml)								
	Level 1		Level 2		Level 3		Level 4		
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	$P_{for\ trend}$*
Men									
Serum ferritin concentration (ng/ml, range)	12·93–102·03		102·07–156·63		156·67–228·98		229·00–1757·68		
No. of subjects	1242		1241		1240		1241		–
No. of carotid IMT	451		416		438		399		–
Model 1†	1·00	Ref.	0·89	0·75, 1·04	0·96	0·81, 1·13	0·83	0·70, 0·98	0·07
Model 2‡	1·00	Ref.	0·97	0·80, 1·17	1·15	0·95, 1·39	0·93	0·77, 1·13	0·76
Model 3§	1·00	Ref.	0·98	0·81, 1·19	1·15	0·95, 1·39	0·91	0·75, 1·10	0·53
Women									
Serum ferritin concentration (ng/ml, range)	5·51–33·07		33·24–56·53		56·55–93·88		93·96–2000·00		
No. of subjects	835		834		834		835		–
No. of carotid IMT	64		139		207		307		–
Model 1†	1·00	Ref.	2·41	1·77, 3·31	3·98	2·97, 5·40	7·01	5·27, 9·44	<0·0001
Model 2‡	1·00	Ref.	1·39	0·98, 1·97	1·45	1·04, 2·03	1·99	1·44, 2·79	<0·0001
Model 3§	1·00	Ref.	1·39	0·98, 1·99	1·39	0·99, 1·97	1·81	1·30, 2·55	<0·001

Ref., referent values.
* Multiple logistic regression analysis.
† Crude model.
‡ Adjusted for age and BMI.
§ Adjusted for age, BMI, smoking status, drinking status, hypertension, hyperlipidaemia, diabetes as well as for family history of CVD, hypertension, hyperlipidaemia and diabetes.

Table 3. Adjusted associations of categories between serum ferritin concentration and prevalence of carotid plaques (Adjusted odds ratios and 95 % confidence intervals)

	Quartiles of serum ferritin concentration (ng/ml)								
	Level 1		Level 2		Level 3		Level 4		
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	$P_{for\ trend}$*
Men									
Serum ferritin concentration (ng/ml, range)	12·93–102·03		102·07–156·63		156·67–228·98		229·00–1757·68		
No. of subjects	1242		1241		1240		1241		–
No. of carotid plaques	344		318		331		287		–
Model 1†	1·00	Ref.	0·90	0·75, 1·08	0·95	0·80, 1·13	0·79	0·66, 0·94	0·02
Model 2‡	1·00	Ref.	0·99	0·82, 1·19	1·13	0·94, 1·37	0·90	0·74, 1·09	0·45
Model 3§	1·00	Ref.	0·99	0·82, 1·21	1·12	0·92, 1·36	0·85	0·69, 1·04	0·17
Women									
Serum ferritin concentration (ng/ml, range)	5·51–33·07		33·24–56·53		56·55–93·88		93·96–2000·00		
No. of subjects	835		834		834		835		–
No. of carotid plaques	72		123		163		243		–
Model 1†	1·00	Ref.	1·83	1·35, 2·51	2·57	1·92, 3·48	4·35	3·29, 5·81	<0·0001
Model 2‡	1·00	Ref.	1·24	0·90, 1·72	1·23	0·89, 1·70	1·73	1·26, 2·38	<0·001
Model 3§	1·00	Ref.	1·24	0·89, 1·73	1·18	0·85, 1·65	1·59	1·15, 2·20	<0·01

Ref., referent values.
* Multiple logistic regression analysis.
† Crude model.
‡ Adjusted for age and BMI.
§ Adjusted for age, BMI, smoking status, drinking status, hypertension, hyperlipidaemia, diabetes as well as for family history of CVD, hypertension, hyperlipidaemia and diabetes.

found a positive association between serum ferritin and carotid atherosclerosis[22,29]. The two remaining studies focusing on IMT suggested a negative association between serum ferritin and carotid atherosclerosis[27,28]. In addition, one cohort study provided evidence for an association between serum ferritin and carotid plaques in an Italian population[21]. IMT was not measured in this analysis[21]. To date, only one small-scale cross-sectional study has been conducted in Asian post-menopausal women[38]. This study suggested that serum ferritin is associated with carotid atherosclerosis[38]. The current study is the first study evaluating the association between serum ferritin concentration and carotid atherosclerosis in an Asian general population, and suggests that increased serum ferritin levels are significantly associated with carotid atherosclerosis in women, but not in men. Nevertheless, results from all studies were inconsistent. The discrepancies among the studies may be partly attributable to the differences in race, dietary habits, sample size and confounding factors. More studies are needed to confirm the exact association between serum ferritin and carotid atherosclerosis.

Evidence suggests that excessive accumulation of Fe promotes atherosclerosis through several putative mechanisms. On the one hand, the proatherogenic properties of Fe are due to its ability to generate ROS[18]. These ROS, in turn, promote LDL peroxidation[18]. The increased oxidative stress induces endothelial dysfunction by decreasing the bioavailability of nitric oxide[39]. Ultimately, atherosclerosis is accelerated as a result of increased platelet activity and leucocyte adhesion[39]. Moreover, owing to ROS, increased expression of the *LOX-1* receptor on endothelial cells leads to mitochondrial DNA damage and autophagy activation[12]. This, in turn, gives rise to an inflammatory response that accelerates atherosclerosis[12]. On the other hand, accumulated Fe in adipocytes leads to adipocyte IR by increasing lipolysis and by decreasing insulin-stimulated glucose transport[20]. Consequently, adipocyte IR promotes a proinflammatory and pro-oxidant state that accelerates atherosclerosis[13].

Animal-based foods are the main sources of Fe[30]. Because Fe content in the Asian dietary pattern is lower than that in the Western dietary pattern[30–32], serum ferritin concentrations in Asian people is expected to be lower than that in Western people. However, serum ferritin concentrations measured in Asian members of our study group were similar to those reported for Western people in other studies[24–26]. Still, the precise reason for the similarity between groups remains unclear. A previous study in North America demonstrated that people of Asian heritage have higher serum ferritin levels than those of White heritage in both men and women[40]. Moreover, a study of haplotype variation among Asians, Europeans and Africans suggested that the *HFE* gene, one of the major regulators of Fe homoeostasis, has differing genetic variants in Europeans and Asians[33]. Thus, we speculate that Asians may tend to have a higher Fe-storage capacity than Westerners. Dietary intakes and genetic factors were not measured in our study. Therefore, further studies are needed to elucidate the issue. On the other hand, the *HFE* genotype (*C282Y* allele) was reported to not be not associated with atherosclerosis, but rather with lower cholesterol in Western populations[41,42]. These studies implied that ferritin might simply be an inflammatory marker for atherosclerosis. However, few studies have evaluated the effects of the *HFE* genotype on the association between ferritin and atherosclerosis in Asian populations. Thus, further studies should be conducted to mechanistically describe the association between ferritin and atherosclerosis.

In the present study, serum ferritin is independently associated with carotid IMT and plaques in women, but not in men. The exact mechanism by which ferritin promotes carotid atherosclerosis in women is still unclear. Menstruation is believed to be an important route for Fe excretion[37]. Menstruation is also considered to be involved in menopause-related diseases[43]. Nevertheless, similar associations were observed when premenopausal and postmenopausal women were analysed separately. As only one cross-sectional study focusing on the association between ferritin and carotid atherosclerosis was conducted in postmenopausal women[38], further studies are required to explore the associations between ferritin and carotid atherosclerosis in premenopausal women and other populations. On the other hand, oestrogen metabolites may regulate the release of Fe^{2+} from ferritin[44], and testosterone has been shown to be significantly negatively associated with ferritin levels in Asian men[45]. Therefore, differences in sex hormones may partly explain the sex-specific association between ferritin and carotid atherosclerosis. However, sex hormones were not measured in our study. Further studies are needed to investigate specific mechanisms underlying sex-specific differences in serum ferritin levels and carotid atherosclerosis.

Several limitations of the present study should be noted. First, this is a cross-sectional study, so we could not infer the causality between serum ferritin and carotid atherosclerosis. Therefore, further cohort studies and intervention trials should be conducted to confirm the association between serum ferritin and carotid atherosclerosis. Second, although numerous confounding factors were adjusted for during the analysis, the study cannot eliminate the potential effects of other unmeasured factors (e.g. dietary intakes and genetic factors) completely. Finally, other Fe status measurements (e.g. total Fe-binding capacity:plasma ferritin concentration ratio) were not measured in this study; thus, further high-quality research is required to verify these results.

Conclusions

The study demonstrated that increased serum ferritin levels are independently associated with IMT and carotid plaques in Asian women, but not in Asian men. These results suggested that serum ferritin might be a risk factor for carotid atherosclerosis in women. Also, these findings will be crucial in disease forecasting in a high-risk population and in developing therapies for carotid atherosclerosis.

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