Original Article

Relationship between ceruloplasmin and oxidative biomarkers including ferritin among healthy Japanese

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Serum ceruloplasmin (CP), a marker relevant to copper metabolism, is one of famous inflammation markers with a reduction in Wilson's disease, whereas serum ferritin is a marker relevant to iron metabolism. Recently, ferritin is pointed out to be related with oxidative stress. However, there is still no population research which showed the relation of CP and ferritin. Therefore, we investigated the relationship between CP and ferritin including oxidative stress biomarkers among healthy Japanese (n = 389). We measured serum CP, ferritin, Fe, high-sensitivity C-reactive protein (hs-CRP), and urinary oxidative stress biomarkers [H_2O_2, 8-hydroxy-2′-deoxyguanosine (8-OHdG), 8-isoprostane] and so on. Subjects showed that age; 41.7 ± 10.0 (year), CP; 31.9 ± 6.8 (mg/dl), ferritin; 123.5 ± 121.0 (ng/ml), hs-CRP; 0.89 ± 2.53 (mg/l), 8-OHdG; 10.2 ± 4.4 [ng/mg creatinine (Cre)] and H_2O_2; 6.5 ± 10.9 (μM/g Cre), (All data mentioned above were expressed as mean ± SD). CP was significantly and positively correlated with hs-CRP and inversely correlated with ferritin, Fe and 8-OHdG. By a multiple logistic regression analysis, odds ratio of CP according to quartiles of hs-CRP was 4.86, and according to quartiles of 8-OHdG was 0.39 after adjusting for age and other confounding factors. In conclusion, our findings suggest that CP was an antioxidative biomarker which controls oxidative stress, whereas ferritin was a marker which may participate in the generation of oxidative stress.

***Key Words*:** **ceruloplasmin, ferritin, 8-OHdG, oxidative stress, high-sensitivity C-reactive protein**

Ceruloplasmin (CP) is a copper-containing glycoprotein of a mean molecular weight of 132 kDa that is detected in human plasma at a concentration of 200 to 400 mg/L. Although its biological roles are not entirely clear, CP has several characteristics such as ferroxidase, copper transport, antioxidant, anti-inflammation, and proinflammatory activity.[1] Ferroxidase activity is associated with anti-oxidative or anti-inflammatory activity by catalyzing the oxidation of iron from the Fe^{2+} ion state to the Fe^{3+} ion state, a crucial step for adequate trans-cellular ionic transport.[2] High levels of CP are associated with atherosclerosis and cardiovascular disease.[3] Moreover, serum copper levels are risk factor for cardiovascular disease.[4,5] Proinflammatory effect of CP is associated with the formation of hydroxyl radical ($^{\bullet}OH$) from Fenton-type reactions of Cu^{2+} with hydrogen peroxide (H_2O_2) and the oxidation of low density lipoprotein. In this reaction, loosely bound copper in CP is involved.[6,7] The serum levels of CP decreased in Wilson's disease, Menkes disease, liver disease, mal-absorption, nutritional copper deficiency, excessive therapeutic zinc administration and aceruloplasminemia. On the contrary, CP increased in malignancy, inflammatory disease, pregnancy, cholestasis, alcoholic liver injury, and diabetes mellitus.[8,9] However, the characteristics of CP in healthy population were not clear. Therefore, we considered that it is quite important to examine the trend of CP in healthy population from the viewpoint of preventive medicine.

Serum ferritin level represents the amount of stored body iron and is regarded as one of the oxidative stress markers by providing Fe^{2+} to the Fenton reaction. The level of serum iron (Fe^{2+}) is well known to decrease in chronic inflammatory diseases.[10] Iron is also involved in oxidative stress by forming $^{\bullet}OH$ from H_2O_2 and Fe^{2+} by the Fenton reaction. Oxidative stress is defined as a situation in which an increased level of reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{-\bullet}$) and H_2O_2, overwhelms the antioxidative defense capacity, resulting in oxidative damage to lipids, DNA and proteins.[11] The 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a product of oxidatively modified DNA base guanine. Urinary 8-OHdG was considered as a sensitive marker that relates with diabetes mellitus,[12] chronic renal failure,[13] and cancer.[14]

We have previously reported that the urinary 8-OHdG was one of useful prospective biomarkers of lifestyle-related disease risks.[15] Still, there is no population research which showed the relation of CP and oxidative stress including high-sensitivity C-reactive protein (hs-CRP), although the research which examined the relation between CP and oxidative stress simultaneously in patients with Behcet's disease.[16,17] Therefore, the present study aimed to examine the relationship between CP and oxidative stress biomarkers (H_2O_2, 8-OHdG, 8-isoprostane, nitrite/nitrate; NOx) and ferritin among healthy Japanese.

Materials and Methods

Subjects. A cross-sectional study concerning the relationship between CP and oxidative stress biomarkers including ferritin was designed within the framework of a laboratory and field study. To examine the characteristics of CP in healthy population, we excluded subjects who had any history of Wilson's disease, cancer, stroke, diabetes, ischemic heart disease or asthma, and who takes any kind of medicines or supplements such as vitamins. Therefore, we finally used health check-up data of 389 healthy Japanese individuals whose serum CP levels were able to be

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measured. All subjects were instructed to fast overnight and not consume any beverage and food except plain water before the measurement. The ethics committee of Okayama University approved the study, and all subjects gave informed consent.

Sampling and measurements. Health assessment was performed from September to December, 2007 by collecting blood samples after overnight fasting for at least 10 h. Serum and plasma samples were preserved at 4°C for the measurement of red blood cell (RBC), white blood cell (WBC), hs-CRP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GTP), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), fasting glucose, fasting insulin, hemoglobin A1c (HbA1c) of NGSP, uric acid (UA) and creatinine (Cre). So, the homeostasis model assessment (HOMA-R) levels were calculated as fasting insulin (μU/ml) × fasting glucose (mg/dl)/405.[18] In addition, ferritin, Fe^{3+} and unsaturated iron-binding capacity (UIBC) were determined in serum samples stored at −80°C until analyses, because there was a time delay until measuring these markers.

Anthropometric measurements were performed according to a standard protocol. Blood pressure (BP) was measured in the morning after 10 min of rest in the sitting position. Abdominal circumference was measured horizontally at the umbilical level at the end of normal expiration by well trained nurses. Body mass index (BMI) was calculated by body weight (kg)/height $(m)^2$ and the subjects whose BMI was 25 and over were diagnosed as obesity according to the criteria for Japanese.[19]

Information on lifestyles including cigarette smoking, alcohol consumption and exercise was obtained by self-reported questionnaires. The Brinkman index [(number of cigarette per day) × (smoking year)] was used to assess smoking status. The amount of alcohol consumption was calculated by assuming one unit was equivalent to 9–12 g of ethanol.[20] The habit of alcohol intake was expressed by drinking frequency and drinking quantity (number of units) per week. The habit of exercise was shown by exercise frequency per week.

Analysis of antioxidant and oxidative stress biomarkers. We used serum CP and urinary H_2O_2, 8-OHdG, 8-isoprostane, and plasma NOx. Serum and plasma samples were stored at −80°C before analysis. Serum CP was analyzed by nephelometry tests. The tests were able to detect 21–37 (mg/dl) of CP. NOx level was determined using an ozone-based chemiluminescence assay.[21]

Urinary oxidative stress biomarkers were determined in spot urine samples stored at −80°C until analysis. Urinary H_2O_2 was analyzed by the ferrous ion oxidation xylenol orange version-1 (FOX-1) assay,[22,23] and the intra-assay and inter-assay coefficients of variation (CV) were 4.3% and 9.7%, respectively. Measurement of 8-OHdG was carried out with an enzyme-linked immunosorbent assay (ELISA) kit from the Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan,[24] and the intra-assay and inter-assay CV were 5.2% and 8.1%, respectively. Møller and Loft[25] indicated that the correlation coefficient of 8-OHdG measurements by ELISA between spot and 24-h urine sample was 0.87. Urinary 8-isoprostane was analyzed using commercially available competitive enzyme immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI),[26] and the intra-assay and inter-assay CV were 5.4% and 11.0%, respectively. Values for H_2O_2, 8-OHdG and 8-isoprostane were normalized by per milligram of Cre measured in urine (Cre test kit, R&D Systems, Minneapolis, MN).

Statistical analysis. The data were expressed as a percentage, the arithmetic mean ± SD values. A statistical analysis was performed using a Mann-Whitney *U* test or unpaired *t* test, two-way analysis of variance (ANOVA) and a multiple logistic regression analysis. Spearman's correlation analysis was performed to examine the relation between CP and the other variables including oxidative stress biomarkers. A multiple logistic regression analysis was performed to test the relationship between CP with 8-OHdG, ferritin and hs-CRP, and between ferritin with 8-OHdG.

A probability value of $p<0.05$ was considered to be statistically significant. Data analyses were performed using SPSS software (SPSS, Chicago, IL; ver. 19.0).

Results

Subject characteristics. The characteristics of the subjects are summarized in Table 1. The obesity (BMI ≥ 25) person was 20.3% ($n = 79$). The group of smoker was 39.8% ($n = 155$), and the group which drinks alcohol 4 times or more per week was 30.8% ($n = 120$). In addition, the proportion of no exercise was 59.9% ($n = 233$). The clinical characteristics by sex are shown in Table 2. The levels of BMI, abdominal circumference, BP, RBC, AST, ALT, γ-GTP, LDL-c, TG, UA, Cre, insulin, Fe, ferritin, 8-isoprostane, and NOx in males were significantly higher than those in females. On the other hand, the levels of HDL-c, CP, hs-CRP, UIBC and 8-OHdG in males were significantly lower than those in females.

Relationship between CP and clinical parameters included oxidative stress. The results of Spearman's correlation analysis between CP and other clinical parameters are presented in Table 3. In all subjects, CP was significantly and positively correlated with BMI, WBC, HbA1c and hs-CRP, but it was significantly and inversely correlated with ferritin, Fe, RBC, Cre, and 8-OHdG. In addition, ferritin was significantly and positively correlated with 8-OHdG. Only in female subjects, CP was significantly and positively correlated with AST, ALT, γ-GTP, UA, NOx, and alcohol consumption.

Table 4 shows the concentration of CP as classified by sex and age groups. By two-way ANOVA, the concentration of CP in male subjects was significantly lower than those in female subjects, although there were no significant differences in the concentration of CP as classified by age groups.

Multiple logistic regression analysis for CP and ferritin. Table 5-1 and 5-2 showed the results by multiple logistic regression analysis about CP or ferritin, according to quartiles of 8-OHdG. The odds ratio of CP according to quartiles of 8-OHdG was 0.39 in all subjects, and 0.29 in male subjects, and 0.20 in female subjects, after adjusted for age and other confounding factors. In addition, *p* value for trend in all subject, and male or female subjects after adjusted for age and other confounding factors were statistically significant. On the other hand, the odds ratio of ferritin according to quartiles of 8-OHdG was 7.39 in all subjects, 3.99 in male subjects, and 57.03 in female subjects, after adjusted for age and other confounding factors. In addition, the trend analysis showed that the odds ratio of CP concentration tended to be low with increasing the urinary concentration of 8-OHdG in all subject, and in male or female subjects after adjusted for age and other confounding factors.

In addition, Table 6 showed the results by multiple logistic regression analysis about CP according to quartiles of ferritin. The odds ratio of CP according to quartiles of ferritin was 0.82 (but was not statistically significant) in all subjects after adjusted for age and other confounding factors. And there were no significant odds ratio after adjusted for age and other related factors in male and female subjects.

Finally, Table 7 and 8 showed the results by multiple logistic regression analysis about CP and ferritin according to quartiles of hs-CRP. The odds ratio of CP according to quartiles of hs-CRP was 4.86 in all subjects, 4.42 in male subjects, and 14.18 in female subjects after adjusted for age and other confounding factors. In addition, the trend analysis showed that there was a significant and positive association between the odds ratio of CP concentration and the quartiles of hs-CRP in all subject, and in male or female subjects after adjusted for age and other confounding

Table 1. Life style profile

n(%)

		All	Male	Female
Total		389 (100.0)	195 (100.0)	194 (100.0)
Age (year)	20–29	50 (12.9)	25 (12.8)	25 (12.9)
	30–39	118 (30.3)	60 (30.8)	58 (29.9)
	40–49	133 (34.2)	69 (35.4)	64 (33.0)
	50–59	71 (18.3)	33 (16.9)	38 (19.6)
	60–69	17 (4.4)	8 (4.1)	9 (4.6)
BMI (kg/m^2)	<18.5	37 (9.5)	13 (6.7)	24 (12.4)
	18.5–24.9	273 (70.2)	135 (69.2)	138 (71.1)
	≥25	79 (20.3)	47 (24.1)	32 (16.5)
Lifestyle				
Smoking	Nonsmoker	234 (60.2)	98 (50.3)	136 (70.1)
	Smoker	155 (39.8)	97 (49.7)	58 (29.9)
Alcohol	No	123 (31.6)	45 (23.1)	78 (40.2)
	3 times or less per week	146 (37.5)	62 (31.8)	84 (43.3)
	4 times or more per week	120 (30.8)	88 (45.1)	32 (16.5)
Exercise	No	233 (59.9)	94 (48.2)	139 (71.6)
	2 times or less per week	107 (27.5)	67 (34.4)	40 (20.6)
	3 times or more per week	49 (12.6)	34 (17.4)	15 (7.7)

Table 2. Clinical characteristics of subjects by sex

Clinical parameter	All (*n* = 389)	Male (*n* = 195)	Female (*n* = 194)	*p* value
Age (year)	41.7 ± 10.0	41.7 ± 9.9	41.7 ± 10.2	0.993
BMI (kg/m^2)	22.9 ± 3.9	23.6 ± 3.6	22.1 ± 3.9	<0.001
Abdominal circumference (cm)	80.6 ± 10.9	83.9 ± 9.7	77.3 ± 11.0	<0.001
Systolic blood pressure (mmHg)	129.8 ± 21.3	133.9 ± 21.2	125.7 ± 20.7	<0.001
Diastolic blood pressure (mmHg)	77.9 ± 14.4	79.9 ± 14.7	75.8 ± 13.8	0.004
Blood profile				
RBC (cell/μl)	468.6 ± 48.0	492.8 ± 48.1	445.2 ± 34.4	<0.001
WBC (cell/μl)	5891.5 ± 1660.6	6036.4 ± 1669.8	5745.9 ± 1642.7	0.065
AST (IU/l)	21.6 ± 9.0	24.0 ± 9.4	19.1 ± 7.8	<0.001
ALT (IU/l)	23.7 ± 19.7	30.1 ± 22.8	17.4 ± 13.2	<0.001
γ-GTP(IU/l)	36.8 ± 63.0	45.4 ± 36.8	28.2 ± 80.4	<0.001
LDL-c (mg/dl)	126.4 ± 35.0	130.2 ± 32.8	122.5 ± 36.8	0.002
HDL-c (mg/dl)	62.3 ± 15.1	56.3 ± 13.0	68.3 ± 14.8	<0.001
TG (mg/dl)	105.6 ± 78.6	125.5 ± 95.1	85.5 ± 50.1	<0.001
Uric acid (mg/dl)	5.19 ± 1.40	6.02 ± 1.10	4.36 ± 1.16	<0.001
Creatinine (mg/dl)	0.73 ± 0.15	0.83 ± 0.11	0.62 ± 0.98	<0.001
Insulin (μU/ml)	5.2 ± 3.2	5.6 ± 3.9	4.9 ± 2.4	0.491
Glucose (mg/dl)	93.7 ± 16.0	97.0 ± 20.0	90.4 ± 9.7	<0.001
HbA1c (%)	4.99 ± 0.55	5.04 ± 0.64	4.94 ± 0.42	0.161
HOMA-R	1.24 ± 0.89	1.37 ± 1.09	1.11 ± 0.61	0.085
Ceruloplasmin (mg/dl)	31.87 ± 6.79	30.20 ± 5.63	33.54 ± 7.43	<0.001
Hs-CRP (mg/l)	0.89 ± 2.53	0.87 ± 1.29	0.91 ± 3.35	<0.001
Fe (μg/dl)	113.9 ± 46.6	119.9 ± 37.5	107.7 ± 53.6	0.001
Ferritin (ng/ml)	123.5 ± 121.0	191.7 ± 130.2	54.7 ± 53.8	<0.001
UIBC (μg/dl)	219.6 ± 69.5	206.2 ± 57.9	233.1 ± 77.4	0.001
NOx (μmol/l)	28.07 ± 15.89	29.53 ± 16.21	26.59 ± 15.47	0.017
Urinary profile				
H_2O_2 (μM/g Cre)	6.51 ± 10.85	5.42 ± 6.14	7.61 ± 14.01	0.367
8-OHdG (ng/mg Cre)	10.16 ± 4.44	9.35 ± 3.66	10.97 ± 5.00	0.001
8-Isoprostane (pg/mg Cre)	781.8 ± 613.1	875.6 ± 603.0	687.5 ± 610.2	<0.001

Each value represents the mean ± SD. Data were analyzed by Mann-Whitney *U* test or unpaired *t* test between male and female.

factors. As to ferritin, the odds ratio in the highest quartile of hs-CRP was 2.18 in all subjects, 2.27 in male subjects, and 0.80 in female subjects after adjusted for age and other confounding factors. The *p* value for trend in all subjects, and male or female subjects after adjusted for age and other confounding factors were not statistically significant. However, the *p* value for trend in all subject was significant ($p = 0.032$) when 8-OHdG was excepted from Model 3 in Table 8 (data not shown).

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Table 3. Spearman's correlation of ceruloplasmin with each parameter

Variable	All (n = 389)		Male (n = 195)		Female (n = 194)	
	r	p	r	p	r	p
Age (year)	0.049	0.334	0.007	0.925	0.088	0.221
BMI (kg/m^2)	0.107	0.034	0.049	0.492	0.279	<0.001
Abdominal circumference (cm)	0.081	0.109	0.048	0.503	0.254	<0.001
Systolic blood pressure (mmHg)	0.008	0.868	0.008	0.914	0.104	0.148
Diastolic blood pressure (mmHg)	0.075	0.140	0.023	0.751	0.184	0.010
Blood profile						
RBC (cell/μl)	–0.109	0.037	0.002	0.979	0.036	0.627
WBC (cell/μl)	0.134	0.008	0.146	0.042	0.184	0.010
AST (IU/l)	0.001	0.979	–0.016	0.824	0.198	0.006
ALT (IU/l)	–0.024	0.644	–0.047	0.511	0.204	0.004
γ-GTP (IU/l)	0.003	0.950	0.070	0.332	0.196	0.006
LDL-c (mg/dl)	0.056	0.274	0.090	0.210	0.105	0.145
HDL-c (mg/dl)	0.055	0.279	–0.035	0.626	–0.035	0.630
TG (mg/dl)	0.059	0.243	0.141	0.050	0.120	0.097
Glucose (mg/dl)	0.060	0.240	0.130	0.071	0.117	0.103
Insulin (μU/ml)	0.067	0.189	0.067	0.353	0.081	0.259
HbA1c (%)	0.115	0.024	0.144	0.045	0.130	0.070
HOMA-R	0.068	0.178	0.079	0.273	0.101	0.161
Uric acid (mg/dl)	–0.049	0.333	0.056	0.438	0.175	0.015
Creatinine (mg/dl)	–0.227	<0.001	–0.108	0.131	–0.086	0.234
Inflammation markers						
Hs-CRP (mg/l)	0.245	<0.001	0.213	0.003	0.361	<0.001
Fe (μg/dl)	–0.130	0.010	–0.169	0.018	–0.028	0.701
Ferritin (ng/ml)	–0.182	<0.001	–0.160	0.026	0.062	0.391
UIBC (μg/dl)	0.204	<0.001	0.216	0.002	0.129	0.074
Oxidative stress markers						
H_2O_2 (μm/g Cre)	0.012	0.819	0.020	0.784	0.006	0.932
8-OHdG (ng/mg Cre)	–0.118	0.020	–0.224	0.002	–0.113	0.116
8-Isoprostane (pg/mg Cre)	–0.003	0.948	0.111	0.122	–0.011	0.883
NOx (μmol/l)	0.034	0.503	–0.022	0.761	0.145	0.044
Lifestyle						
Smoking value	0.040	0.426	0.092	0.199	0.102	0.158
Alcohol consumption	0.040	0.428	0.077	0.287	0.148	0.039
Exercise	–0.086	0.089	–0.017	0.815	–0.045	0.533

Table 4. Effect of sex and age on ceruloplasmin

Groups		Age					Two-way ANOVA		
		20–29	30–39	40–49	50–59	60–69			
All	(n = 389)	(n = 50)	(n = 118)	(n = 133)	(n = 71)	(n = 17)			
Male	(n = 195)	(n = 25)	(n = 60)	(n = 69)	(n = 33)	(n = 8)	Main effects		Interaction
Female	(n = 194)	(n = 25)	(n = 58)	(n = 64)	(n = 38)	(n = 9)	Sex (S)	Age (A)	(S × A)
							p<0.001	p = 0.486	p = 0.348
All	31.87 ± 6.79	30.69 ± 6.57	31.91 ± 6.02	32.18 ± 6.45	31.57 ± 8.22	33.78 ± 8.53			
Male	30.20 ± 5.63	29.16 ± 4.44	30.36 ± 5.03	31.16 ± 5.65	28.82 ± 6.12	29.66 ± 9.72			
Female	33.54 ± 7.43	32.22 ± 7.97	33.51 ± 6.57	33.27 ± 7.10	33.96 ± 9.09	37.44 ± 5.55			

Each value represents the mean ± SD. Data were analyzed by two-way ANOVA (sex and age as main effects).

Discussion

Inverse association of CP with ferritin was observed in healthy population of this study, although CP and ferritin were positively associated with hs-CRP, a biomarker of inflammatin. The same relationship between CP, ferritin and hs-CRP was also observed in amyotrophic lateral sclerosis (ALS) patients.[27] In ALS, increased levels of serum ferritin may contribute to the etiology. Lower levels of CP might contribute to the pathogenesis of alzhaimer's disease although an inverse relation between CP and ferritin was observed not only in alzheimer's disease but also in healthy population.[28] However, these previously reports were based on the result by univariate analysis. The inverse association of CP with ferritin in the present study was robust in healthy population by multivariate statistics. Moreover, this study demonstrated that CP was inversely associated with urinary 8-OHdG, a biomarker of oxidative stress, and then ferritin was positively associated with 8-OHdG. Therefore, it is speculated that CP may act antioxidatively against oxidative stress induced by ferritin.

Ferritin is an iron binding protein that can store Fe^{3+} ions and

Table 5-1. Odds ratio of ceruloplasmin according to quartiles of 8-OHdG

	Quartiles of 8-OHdG				*p* for trend
	Q1	Q2	Q3	Q4	
All (*n* = 389)					
Model 1	1.00	0.73 (0.41–1.32)	0.70 (0.39–1.26)	0.55 (0.31–0.98)	0.047
Model 2	1.00	0.75 (0.41–1.36)	0.58 (0.31–1.07)	0.41 (0.22–0.76)	0.004
Model 3	1.00	0.78 (0.41–1.50)	0.62 (0.32–1.19)	0.39 (0.19–0.80)	0.009
Male (*n* = 195)					
Model 1	1.00	0.67 (0.29–1.57)	0.41 (0.17–0.97)	0.31 (0.13–0.73)	0.004
Model 4	1.00	0.71 (0.28–1.77)	0.39 (0.15–1.02)	0.29 (0.11–0.78)	0.007
Female (*n* = 194)					
Model 1	1.00	0.81 (0.36–1.84)	0.74 (0.33–1.68)	0.53 (0.23–1.19)	0.128
Model 4	1.00	0.69 (0.26–1.86)	0.56 (0.21–1.49)	0.20 (0.63–0.65)	0.008

Data were analyzed by multiple logistic regression analysis. Data in pareutheses were 95% CI. Model 1: Not adjusted. Model 2: Adjusted for sex and age. Model 3: Adjusted for age, sex, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, Hs-CRP, NOx, ferritin, UA, Cre, Smoking, Alcohol and Exercise. Model 4: Adjusted for age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, Hs-CRP, NOx, ferritin, UA, Cre, Smoking, Alcohol and Exercise.

Table 5-2. Odds ratio of ferritin according to quartiles of 8-OHdG

	Quartiles of 8-OHdG				*p* for trend
	Q1	Q2	Q3	Q4	
All (*n* = 389)					
Model 1	1.00	1.78 (0.99–3.20)	0.95 (0.53–1.71)	1.48 (0.83–2.63)	0.560
Model 2	1.00	2.03 (0.85–4.82)	1.84 (0.75–4.53)	5.76 (2.24–14.8)	0.001
Model 3	1.00	2.59 (0.96–7.01)	2.37 (0.84–6.67)	7.39 (2.39–22.9)	0.001
Male (*n* = 195)					
Model 1	1.00	2.49 (1.06–5.82)	1.54 (0.66–3.63)	2.49 (1.06–5.82)	0.099
Model 4	1.00	3.10 (1.18–8.16)	1.73 (0.62–4.80)	3.99 (1.36–11.71)	0.040
Female (*n* = 194)					
Model 1	1.00	8.99 (3.01–26.9)	11.77 (3.92–35.32)	36.31 (11.19–117.83)	<0.001
Model 4	1.00	18.73 (4.79–73.28)	17.16 (4.43–66.44)	57.03 (12.86–252.96)	<0.001

Data were analyzed by multiple logistic regression analysis. Data in pareutheses were 95% CI. Model 1: Not adjusted. Model 2: Adjusted for sex and age. Model 3: Adjusted for sex, age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, Hs-CRP, NOx, CP, UA, Cre, Smoking, Alcohol and Exercise. Model 4: Adjusted for age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, Hs-CRP, NOx, CP, UA, Cre, Smoking, Alcohol and Exercise.

Table 6. Odds ratio of ceruloplasmin according to quartiles of ferritin

	Quartiles of Ferritine				*p* for trend
	Q1	Q2	Q3	Q4	
All (*n* = 389)					
Model 1	1.00	0.93 (0.52–1.66)	0.74 (0.42–1.32)	0.50 (0.28–0.89)	0.014
Model 2	1.00	1.01 (0.56–1.84)	1.18 (0.57–2.45)	0.83 (0.38–1.82)	0.756
Model 3	1.00	1.16 (0.58–2.31)	1.34 (0.57–3.19)	0.82 (0.32–2.12)	0.788
Male (*n* = 195)					
Model 1	1.00	0.55 (0.24–1.28)	0.50 (0.22–1.17)	0.46 (0.20–1.07)	0.076
Model 4	1.00	0.57 (0.23–1.43)	0.53 (0.21–1.36)	0.49 (0.18–133)	0.169
Female (*n* = 194)					
Model 1	1.00	1.10 (0.49–2.46)	1.14 (0.50–2.60)	1.42 (0.62–3.22)	0.412
Model 4	1.00	1.25 (0.47–3.31)	1.02 (0.36–2.91)	1.55 (0.48–4.98)	0.562

Data were analyzed by multiple logistic regression analysis. Data in pareutheses were 95% CI. Model 1: Not adjusted. Model 2: Adjusted for sex and age. Model 3: Adjusted for age,sex, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, 8-OHdG, NOx, Hs-CRP, UA, Cre, Smoking, Alcohol and Exercise. Model 4: Adjusted for age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, 8-OHdG, NOx, Hs-CRP, UA, Cre, Smoking, Alcohol and Exercise.

is distributed in the whole body. Serum levels of ferritin are considered to reflect body iron store.[29] Although two opposite functions such as an antioxidative function by its chelating effect of free iron[30] and a promotor function for oxidative stress by releasing free iron[31] were reported, there are many epidemiological evidences to show a positive association of ferritin with urinary 8-OHdG.[32–34] This study showed a positive association of ferritin with urinary 8-OHdG, indicating its promotion of oxidative stress.

CP is known as an acute phase protein which increases 2- or 3-fold in inflammatory conditions. However, it has contradictory functions between pro-inflammation and anti-oxidation. The

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Table 7. Odds ratio of ceruloplasmin according to quartiles of hs-CRP

	Quartiles of hs-CRP				*p* for trend
	Q1	Q2	Q3	Q4	
All (*n* = 389)					
Model 1	1.00	1.46 (0.81–2.66)	1.56 (0.87–2.78)	3.99 (2.17–7.32)	<0.001
Model 2	1.00	1.67 (0.90–3.10)	1.91 (1.04–3.53)	5.39 (2.81–10.34)	<0.001
Model 3	1.00	1.55 (0.80–3.00)	2.02 (1.01–4.04)	4.86 (2.16–10.92)	<0.001
Male (*n* = 195)					
Model 1	1.00	1.23 (0.53–2.87)	1.06 (0.46–2.42)	3.11 (1.32–7.33)	0.018
Model 4	1.00	0.99 (0.38–2.59)	1.28 (0.45–3.63)	4.42 (1.38–14.14)	0.014
Female (*n* = 194)					
Model 1	1.00	1.34 (0.56–3.23)	3.68 (1.57–8.63)	7.08 (2.87–17.49)	<0.001
Model 4	1.00	1.48 (0.54–4.09)	6.53 (2.16–19.76)	14.18 (3.91–51.41)	<0.001

Data were analyzed by multiple logistic regression analysis. Data in pareutheses were 95% CI. Model 1: Not adjusted. Model 2: Adjusted for sex and age. Model 3: Adjusted for age, sex, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, 8-OHdG, NOx, ferritin, UA, Cre, Smoking, Alcohol and Exercise. Model 4: Adjusted for age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, 8-OHdG, NOx, ferritin, UA, Cre, Smoking, Alcohol and Exercise.

Table 8. Odds ratio of ferritin according to quartiles of Hs-CRP

	Quartiles of Hs-CRP				*p* for trend
	Q1	Q2	Q3	Q4	
All (*n* = 389)					
Model 1	1.00	1.39 (0.76–2.53)	2.86 (1.59–5.15)	3.41 (1.87–6.21)	<0.001
Model 2	1.00	0.88 (0.38–2.04)	2.10 (0.93–4.75)	2.72 (1.18–6.28)	0.004
Model 3	1.00	0.83 (0.32–2.21)	2.41 (0.91–6.42)	2.18 (0.67–7.08)	0.076
Male (*n* = 195)					
Model 1	1.00	1.12 (0.47–2.62)	3.01 (1.29–7.00)	3.09 (1.32–7.27)	0.001
Model 4	1.00	1.06 (0.38–2.94)	3.59 (1.21–10.65)	2.27 (0.67–7.72)	0.066
Female (*n* = 194)					
Model 1	1.00	3.15 (1.34–7.43)	3.34 (1.44–7.75)	2.47 (1.07–5.72)	0.040
Model 4	1.00	2.28 (0.76–6.83)	0.92 (0.28–2.97)	0.80 (0.21–3.05)	0.477

Data were analyzed by multiple logistic regression analysis. Data in pareutheses were 95% CI. Model 1: Not adjusted. Model 2: Adjusted for sex and age. Model 3: Adjusted for sex, age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, 8-OHdG, NOx, CP, UA, Cre, Smoking, Alcohol and Exercise. Model 4: Adjusted for age, BMI, Systolic blood pressure, RBC, WBC, ALT, LDL-c, HOMA-R, HbA1c, 8-OHdG, NOx, CP, UA, Cre, Smoking, Alcohol and Exercise.

increase in CP was considered to be a risk factor for cardiovascular disease,[35] which is associated with copper ion-related oxidative stress and augmented oxidative stress by nitric oxide consumption of CP. However, according to the relationship between diabetes mellitus and CP, the changes for CP were inconsistent.[36–38] In this inconsistency, opposite functions such as copper ion related oxidative stress and antioxidative function related to ferroxidase are involved in the pathophysiology of diabetes mellitus. In this study, CP was inversely associated with urinary 8-OHdG in all subjects and as well in male subjects. On the other hand, the level of plasma NOx in only female subjects was significantly and positively correlated with serum CP. However, urinary 8-isoprostane and H_2O_2 were not significantly correlated with serum CP. Moreover, the level of NOx was significantly and positively correlated with hs-CRP in all subjects (data not shown). Although the concentration of hs-CRP was not high enough, the subjects with the high level of hs-CRP also showed higher level of NOx and CP among the healthy subjects in our study. So, serum CP may prevent the oxidative stress and reduce the levels of Fe^{2+} and 8-OHdG among the healthy subjects. In healthy population study, the result of this study is first evidence to show antioxidative function of CP in association with ferritin.

Several limitations of the study should be noted. First, causal relationships could not be determined because this study was a cross-sectional study. Second, some reporting bias may have been introduced because the information on lifestyle habits like smoking and drinking was obtained via self-reported questionnaires. Third, sample size of this research was not large enough, and also we evaluated only in healthy subjects. Therefore, clear relationships between CP and parameters may not be noted in this study. Further studies with large sample size including patients with the high level CP and/or hs-CRP and longitudinal examination are required to confirm the antioxidative function of the serum CP.

In conclusion, our findings suggest that CP was an antioxidative biomarker controls oxidative stress, whereas ferritin was a marker which may participate in the generation of oxidative stress.

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Abbreviations

ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase

BMI	body mass index
BP	blood pressure
CP	ceruloplasmin
Cre	creatinine
CV	coefficients of variation
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FOX-1	ferrous ion oxidation xylenol orange version-1
γ-GTP	γ-glutamyltranspeptidase
HbA1c	hemoglobin A1c
HDL-c	high-density lipoprotein-cholesterol
H_2O_2	hydrogen peroxide
HOMA-R	homeostasis model assessment
hs-CRP	high-sensitivity C-reactive protein
LDL-c	low-density lipoprotein-cholesterol
NOx	nitrite/nitrate
$O_2^{-\bullet}$	superoxide anion radical
$^{\bullet}OH$	hydroxyl radical
8-OHdG	8-hydroxy-2'-deoxyguanosine
RBC	red blood cell
ROS	reactive oxygen species
TG	triglycerides
UA	uric acid
UIBC	unsaturated iron-binding capacity
WBC	white blood cell

Conflict of Interest

No potential conflicts of interest were disclosed.

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