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Original Article

Serum levels of trace elements and vitamins in coronary artery disease and their predictive values

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Abstract: *Background:* The traditional risk factors of coronary artery disease (CAD) account for only 50% of the total risk of CAD. In addition to traditional risk factors, abnormal levels of trace elements and vitamins in human serum may be associated with CAD according to recent studies. However, the relationship between serum levels of trace elements and vitamins and CAD has still not been comprehensively analyzed, and their values in predicting CAD has still not been evaluated. *Objective:* To identify the independent risk factors of CAD and evaluate their values in predicting CAD. *Methods:* Serum levels of trace elements and vitamins were measured in 198 CAD patients and 396 healthy controls matched to these cases by gender and age at a ratio of 1:2. Univariate analysis was performed using chi-square test, and multivariate analysis was then performed with a backward stepwise logistic regression model for the variables with a *P* value less than 0.10 in univariate analysis. Predictive values of identified independent risk factors were evaluated with receiver operating characteristic (ROC) curve. *Results:* Univariate analysis showed that the serum levels of Fe, Cu, Cr and Pb were higher in CAD patients than in healthy controls ($P<0.001$), and the serum levels of Zn, Se, vitamin D_3, vitamin B_{12} and FA were lower in CAD patients than in healthy controls ($P<0.001$). The serum levels of Mn, Co, Ni, V, vitamin A, vitamin C and vitamin E were not statistically different between CAD patients and healthy controls ($P>0.05$). Multivariate analysis showed that the serum level of Fe>1.71 mg/L, Cu>0.67 mg/L, Zn≤1.53 mg/L, Se≤1.10 mg/L, Pb>0.067 mg/L, vitamin D_3≤36.47 μg/dL, vitamin B_{12}≤68.41 μg/dL and FA≤13.57 μg/dL were independent risk factors of CAD. According to the area under curve (AUC), the serum level of Fe>1.71 mg/L, Zn≤1.08 mg/L, Se≤0.67 mg/L, vitamin B_{12}≤68.41 μg/dL and FA≤13.57 μg/dL had moderate values (AUC>0.700) when applied in predicting CAD. *Conclusions:* Elevated serum levels of Fe, Cu and Pb and decreased serum levels of Zn, Se, vitamin D_3, vitamin B_{12} and FA were independent risk factors of CAD, and moreover the serum levels of Fe, Zn, Se, vitamin B_{12} and FA could be applied in predicting CAD.

Keywords: Coronary artery disease, trace elements, vitamins, risk factors, predictive values

Introduction

Coronary artery disease (CAD), predominantly resulted from atherosclerosis with endothelial dysfunction, severely threatens human health around the world [1, 2]. It is still a major challenge for public health despite all-round efforts in its prevention and management [3]. In China, CAD has an alarming increasing incidence in the past two decades and is predicted to be the main cause of death in the next decade. The traditional risk factors of CAD include smoking, family history, hyperlipidemia, hypertension, diabetes mellitus, serum cholesterol etc, however, these risk factors account for only 50% of the total risk of CAD [4, 5]. In addition to these traditional, most recognized risk factors, new risk factors are emerging with potential relevant diagnostic and therapeutic implications. Studies show that abnormal levels of trace elements and vitamins in human serum may be associated with CAD [6-8]. However, the relationship between serum levels of trace elements and vitamins and CAD has still not been comprehensively analyzed, and their values in predicting CAD has still not been evaluated. In this paper, the serum levels of eight trace elements and six vitamins were measured and analyzed for CAD and non-CAD patients, and the aim was to identify the independent risk factors of CAD and evaluate their values in predicting CAD.

Materials and methods

Participants

In this single-center case-control study, a total of 198 CAD patients, diagnosed by two experi-

Table 1. Concentrations of serum trace elements of case group and control group

	Case group (n=198)			Control group (n=396)		
	Median	IQR	*P**	Median	IQR	*P**
Fe (mg/L)	3.890	1.710	0.018	1.710	1.850	0.015
Cu (mg/L)	0.860	0.690	0.027	0.670	0.610	0.025
Zn (mg/L)	0.900	0.570	0.031	1.080	0.660	0.029
Mn (mg/L)	0.036	0.026	0.037	0.034	0.029	0.041
Se (mg/L)	0.440	0.340	0.033	0.670	0.680	0.035
Cr (mg/L)	0.122	0.129	0.029	0.091	0.080	0.030
Co (mg/L)	0.139	0.214	0.038	0.127	0.214	0.040
Pb (mg/L)	0.132	0.102	0.013	0.097	0.075	0.022
Ni (mg/L)	0.133	0.073	0.042	0.122	0.068	0.036
V (mg/L)	0.177	0.073	0.026	0.186	0.078	0.034

*: Kolmogorov-Smirnov test.

Table 2. Comparison of serum levels of trace elements between case group and control group

	Control group (n=396)	Case group (n=198)	χ^2	*P*
Fe (mg/L)			182.678	<0.001
Grade 1: ≤Q1 (0.95)	99	3		
Grade 2: >Q1 and ≤Q2 (1.71)	99	5		
Grade 3: >Q2 and ≤Q3 (2.80)	99	29		
Grade 4: >Q3	99	161		
Cu (mg/L)			30.04	<0.001
Grade 1: ≤Q1 (0.31)	99	19		
Grade 2: >Q1 and ≤Q2 (0.67)	99	36		
Grade 3: >Q2 and ≤Q3 (0.92)	99	68		
Grade 4: >Q3	99	75		
Zn (mg/L)			107.288	<0.001
Grade 1: ≤Q1 (0.87)	99	95		
Grade 2: >Q1 and ≤Q2 (1.08)	99	89		
Grade 3: >Q2 and ≤Q3 (1.53)	99	11		
Grade 4: >Q3	99	3		
Mn (mg/L)			0.67	0.88
Grade 1: ≤Q1 (0.017)	99	44		
Grade 2: >Q1 and ≤Q2 (0.034)	99	53		
Grade 3: >Q2 and ≤Q3 (0.046)	99	52		
Grade 4: >Q3	99	49		
Se (mg/L)			105.033	<0.001
Grade 1: ≤Q1 (0.42)	99	96		
Grade 2: >Q1 and ≤Q2 (0.67)	99	87		
Grade 2: >Q2 and ≤Q3 (1.10)	99	12		
Grade 2: >Q3	99	3		
Cr (mg/L)			47.125	<0.001
Grade 1: ≤Q1 (0.050)	99	14		
Grade 2: >Q1 and ≤Q2 (0.091)	99	29		
Grade 3: >Q2 and ≤Q3 (0.130)	99	72		
Grade 4: >Q3	99	83		

enced cardiologists according to American Heart Association guidelines and coronary angiography in the Second Department of Cardiology in Heze Municipal Hospital, were assigned to the case group. In the same period, 396 healthy controls were matched to these cases by gender and age at a ratio of 2:1 in the Center of Physical Examination. CAD patients with following diseases were excluded from this study: cancers, blood diseases, malnutrition, pregnant women, renal insufficiency, liver diseases, thyroid dysfunction, hyperthyroidism, and receiving hormone drugs and products containing trace elements and vitamins.

This study received the approval of the ethic committee of Heze Municipal Hospital (2012080516), and each participant provided signed informed consent.

Laboratory methods

Blood samples were collected into the royal blue stoppered trace element tubes (catalog #369737, BD Vacutainer™ glass sterile tube,

Co (mg/L)			7.071	0.07
Grade 1: ≤Q1 (0.090)	99	37		
Grade 2: >Q1 and ≤Q2 (0.127)	99	40		
Grade 3: >Q2 and ≤Q3 (0.304)	99	56		
Grade 4: >Q3	99	65		
Pb (mg/L)			42.161	<0.001
Grade 1: ≤Q1 (0.067)	99	13		
Grade 2: >Q1 and ≤Q2 (0.097)	99	39		
Grade 3: >Q2 and ≤Q3 (0.142)	99	58		
Grade 4: >Q3	99	88		
Ni (mg/L)			5.371	0.147
Grade 1: ≤Q1 (0.070)	99	38		
Grade 2: >Q1 and ≤Q2 (0.122)	99	42		
Grade 3: >Q2 and ≤Q3 (0.138)	99	55		
Grade 4: >Q3	99	63		
V (mg/L)			5.116	0.163
Grade 1: ≤Q1 (0.136)	99	62		
Grade 2: >Q1 and ≤Q2 (0.186)	99	56		
Grade 3: >Q2 and ≤Q3 (0.214)	99	41		
Grade 4: >Q3	99	39		

Table 3. Concentrations of serum vitamins of case group and control group

	Case group			Control group		
	Median	IQR	*P**	Median	IQR	*P**
Vitamin A (μg/dL)	53.29	54.60	0.019	53.65	54.90	0.021
Vitamin C (μg/dL)	921.00	887.00	0.023	946.00	892.00	0.018
Vitamin D_3 (μg/dL)	30.14	19.96	0.021	36.47	22.84	0.026
Vitamin E (μg/dL)	1.01	0.72	0.015	0.90	0.89	0.017
Vitamin B_{12} (μg/dL)	49.64	39.01	0.029	68.41	53.72	0.037
FA (μg/dL)	10.29	7.82	0.027	13.57	6.77	0.033

*: Kolmogorov-Smirnov test.

Becton Dickinson Co., Franklin Lakes, NJ, USA) from the antecubital vein after an overnight fasting in all participants, and serums were then separated within 2 hours and stored at -80°C until analysis. The concentrations of serum iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), chromium (Cr), cobalt (Co), lead (Pb), nickel (Ni) and vanadium (V) were measured with an Agilent 7500ce inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, Inc., Tokyo, Japan) following 1:20 dilutions of 100 μL of serum with diluent containing 0.5% (v/v) HNO3 [9]. All standards were from National Research Center for Certified Reference Material (NRCCRMS), and all water used for the analysis was deionized and double distilled. Serum vitamin B_{12} and folic acid (FA) were measured with enzyme-linked immunoabsorbent assay (ELISA), and vitamin A, C, D_3 and E were measured with high performance liquid chromatography (HPLC).

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 for Windows (SPSS Inc., USA). The normality of the distributions for quantitative variables was checked with Kolmogorov-Smirnov test. Median and interquartile range (IQR) were employed to describe central tendency and dispersion for non-normal data, respectively. The serum levels of vitamins and trace elements were grouped into 4 grades based on the Q1, Q2 and Q3 of controls, and the cutoffs were then applied to the cases. Univariate analysis was performed using chi-square test. Multivariate analysis was then performed with a backward stepwise logistic regression model for the variables with a *P* value less than 0.10 in univariate analysis. Predictive value of independent risk factors was evaluated with receiver operating characteristic (ROC) curve. Significance was set at $P<0.05$.

Results

Univariate analysis of serum trace elements

The concentrations of serum Fe, Cu, Zn, Mn, Se, Cr, Co, Pb, Ni and V showed skewed distributions. Therefore, they were described with median and IQR (**Table 1**).

The serum levels of trace elements were grouped into 4 grades (Grade 1: ≤Q1, Grade 2: >Q1 and ≤Q2, Grade 3: >Q2 and ≤Q3, Grade 4: >Q3)

Table 4. Comparison of serum levels of vitamins between case group and control group

	Control group (n=396)	Case group (n=198)	χ^2	*P*
Vitamin A (μg/dL)			0.468	0.926
Grade 1: ≤Q1 (34.1)	99	54		
Grade 2: >Q1 and ≤Q2 (53.65)	99	50		
Grade 3: >Q2 and ≤Q3 (89.00)	99	48		
Grade 4: >Q3	99	46		
Vitamin C (μg/dL)			0.915	0.822
Grade 1: ≤Q1 (655)	99	56		
Grade 2: >Q1 and ≤Q2 (946)	99	50		
Grade 3: >Q2 and ≤Q3 (1547)	99	47		
Grade 4: >Q3	99	45		
Vitamin D_3 (μg/dL)			31.786	<0.001
Grade 1: ≤Q1 (29.67)	99	76		
Grade 2: >Q1 and ≤Q2 (36.47)	99	68		
Grade 3: >Q2 and ≤Q3 (52.51)	99	36		
Grade 2: >Q3	99	18		
Vitamin E (μg/dL)			3.939	0.268
Grade 1: ≤Q1 (0.67)	99	39		
Grade 2: >Q1 and ≤Q2 (0.90)	99	44		
Grade 3: >Q2 and ≤Q3 (1.56)	99	54		
Grade 4: >Q3	99	61		
Vitamin B_{12} (μg/dL)			35.529	<0.001
Grade 1: ≤Q1 (49.57)	99	95		
Grade 2: >Q1 and ≤Q2 (68.41)	99	86		
Grade 3: >Q2 and ≤Q3 (103.29)	99	13		
Grade 4: >Q3	99	4		
FA (μg/dL)			34.928	<0.001
Grade 1: ≤Q1 (10.28)	99	98		
Grade 2: >Q1 and ≤Q2 (13.57)	99	89		
Grade 3: >Q2 and ≤Q3 (17.05)	99	9		
Grade 2: >Q3	99	2		

FA: Folic acid.

based on the Q1, Q2 and Q3 of controls, and the cutoffs were then applied to the cases. According to the results of univariate analysis (**Table 2**), the serum levels of Fe, Cu, Cr and Pb were higher in the case group than in the control group (*P*<0.001), and the serum levels of Zn and Se were lower in the case group than in the control group (*P*<0.001). The serum levels of Mn, Co, Ni and V were not statistically different between the case and control group (*P*>0.05).

Univariate analysis of serum vitamins

The concentrations of serum vitamin A, C, D_3, E, B_{12} and FA also showed skewed distributions. Therefore, they were described with median and IQR (**Table 3**).

The serum levels of vitamins were also grouped into 4 grades (Grade 1: ≤Q1, Grade 2: >Q1 and ≤Q2, Grade 3: >Q2 and ≤Q3, Grade 4: >Q3) based on the Q1, Q2 and Q3 of controls, and the cutoffs were then applied to the cases. According to the results of univariate analysis (**Table 4**), the serum levels of vitamin D_3, B_{12} and FA were lower in the case group than in the control group (*P*<0.001). The serum levels of vitamin A, C and E were not statistically different between the case and control group (*P*>0.05).

Multivariate analysis

The serum levels of Fe, Cu, Zn, Se, Cr, Co, Pb, vitamin D_3, vitamin B_{12} and FA were included in

Table 5. Identified independent risk factors for CAD

	Regression coefficient	Standard error	Wald	*OR*	95% *CI*	*P*
Fe (mg/L)			32.417			<0.001
Grade 2	0.235	0.164	4.612	1.574	0.287-3.079	0.116
Grade 3	0.483	0.351	15.087	8.956	1.582-16.379	<0.001
Grade 4	0.614	0.492	51.369	26.385	2.413-45.584	<0.001
Cu (mg/L)			7.482			0.006
Grade 2	0.217	0.161	4.308	1.494	0.193-3.018	0.127
Grade 3	0.389	0.186	9.472	4.016	1.125-11.374	0.003
Grade 4	0.423	0.272	11.379	4.438	1.132-13.096	<0.001
Zn (mg/L)			27.159			<0.001
Grade 1	0.529	0.402	25.548	19.438	2.197-34.139	<0.001
Grade 2	0.517	0.385	19.184	17.047	2.105-30.262	<0.001
Grade 3	0.218	0.169	8.368	3.426	1.104-9.385	0.005
Se (mg/L)			26.863			<0.001
Grade 1	0.531	0.404	25.552	19.44	2.198-34.146	<0.001
Grade 2	0.515	0.382	19.098	16.962	2.094-29.179	<0.001
Grade 3	0.221	0.172	8.371	3.445	1.105-9.389	0.005
Pb (mg/L)			12.574			<0.001
Grade 2	0.284	0.185	6.974	2.916	1.089-7.126	0.012
Grade 3	0.392	0.218	10.036	5.327	1.133-12.039	0.001
Grade 4	0.439	0.285	16.483	6.926	1.147-14.182	<0.001
Vitamin D_3 (μg/dL)			11.482			<0.001
Grade 1	0.319	0.295	14.748	4.239	1.116-10.135	<0.001
Grade 2	0.307	0.274	9.261	3.574	1.108-9.017	0.004
Grade 3	0.258	0.183	4.723	1.904	0.279-4.176	0.109
Vitamin B_{12} (μg/dL)			17.591			<0.001
Grade 1	0.502	0.397	19.637	11.758	1.217-20.539	<0.001
Grade 2	0.496	0.382	14.359	11.054	1.207-20.165	<0.001
Grade 3	0.341	0.306	5.072	3.228	0.294-8.271	0.089
FA (μg/dL)			18.648			<0.001
Grade 1	0.528	0.452	20.114	12.847	1.285-21.846	<0.001
Grade 2	0.513	0.435	15.268	12.137	1.274-21.016	<0.001
Grade 3	0.356	0.309	5.218	3.839	0.442-9.382	0.078

CAD: Coronary artery disease; FA: Folic acid.

multivariate analysis according to the results of univariate analysis. Multivariate analysis showed that the serum level of Fe>1.71 mg/L, Cu>0.67 mg/L, Zn≤1.53 mg/L, Se≤1.10 mg/L, Pb>0.067 mg/L, vitamin D_3≤36.47 μg/dL, vitamin B_{12}≤68.41 μg/dL and FA≤13.57 μg/dL were independent risk factors of CAD (**Table 5**).

Predictive values of identified independent risk factors

The results of Fe, Cu, Zn, Se, Pb, vitamin D_3, vitamin B_{12} and FA applied in predicting CAD were shown in **Table 6**, and their predictive values were then evaluated with ROC curve. According to the area under curve (AUC), the serum level of Fe>1.71 mg/L, Zn≤1.08 mg/L, Se≤0.67 mg/L, vitamin B_{12}≤68.41 μg/dL and FA≤13.57 μg/dL had moderate values (AUC> 0.700) when applied in predicting CAD (**Figure 1**).

Discussion

Trace elements and vitamins play an important role in various physiological and metabolic processes. Their abnormal concentrations are often associated with many pathological condi-

Table 6. Results of Fe, Cu, Zn, Se, Pb, vitamin D_3, vitamin B_{12} and FA applied in predicting CAD

	Cutoffs	Predictive standard	Golden standard	
			CAD (n=198)	Non-CAD (n=396)
Fe (mg/L)	Q2 (1.71)	>1.71 (Positive)	190	198
		≤1.71 (Negative)	8	198
	Q3 (2.80)	>2.80 (Positive)	161	99
		≤2.80 (Negative)	37	297
Cu (mg/L)	Q2 (0.67)	>0.67 (Positive)	143	198
		≤0.67 (Negative)	55	198
	Q3 (0.92)	>0.92 (Positive)	75	99
		≤0.92 (Negative)	123	297
Zn (mg/L)	Q1 (0.87)	≤0.87 (Positive)	95	99
		>0.87 (Negative)	103	297
	Q2 (1.08)	≤1.08 (Positive)	184	198
		>1.08 (Negative)	14	198
	Q3 (1.53)	≤1.53 (Positive)	195	297
		>1.53 (Negative)	3	99
Se (mg/L)	Q1 (0.42)	≤0.42 (Positive)	96	99
		>0.42 (Negative)	102	297
	Q2 (0.67)	≤0.67 (Positive)	183	198
		>0.67 (Negative)	15	198
	Q3 (1.10)	≤1.10 (Positive)	195	297
		>1.10 (Negative)	3	99
Pb (mg/L)	Q1 (0.067)	>0.067 (Positive)	185	297
		≤0.067 (Negative)	13	99
	Q2 (0.097)	>0.097 (Positive)	146	198
		≤0.097 (Negative)	52	198
	Q3 (0.142)	>0.142 (Positive)	88	99
		≤0.142 (Negative)	110	297
Vitamin D_3 (μg/dL)	Q1 (29.67)	≤29.67 (Positive)	76	99
		>29.67 (Negative)	122	297
	Q2 (36.47)	≤36.47 (Positive)	144	198
		>36.47 (Negative)	54	198
Vitamin B_{12} (μg/dL)	Q1 (49.57)	≤49.57 (Positive)	95	99
		>49.57 (Negative)	103	297
	Q2 (68.41)	≤68.41 (Positive)	181	198
		>68.41 (Negative)	17	198
FA (μg/dL)	Q1 (10.28)	≤10.28 (Positive)	98	99
		>10.28 (Negative)	100	297
	Q2 (13.57)	≤13.57 (Positive)	187	198
		>13.57 (Negative)	11	198

CAD: Coronary artery disease; FA: Folic acid.

tions, which may eventually lead to the occurrence of diseases [10]. Some trace elements have been reported to be risk factors of CAD [6, 7, 11], and meanwhile several antioxidant vitamins have been reported to be protective factors of CAD [12-15].

Studies show that the disruption of elemental homeostasis, especially for redox-sensitive elements, can cause elemental-mediated formation of deleterious free radicals [16]. As members of redox-sensitive elements, Fe and Cu can be toxic when present in excess. The

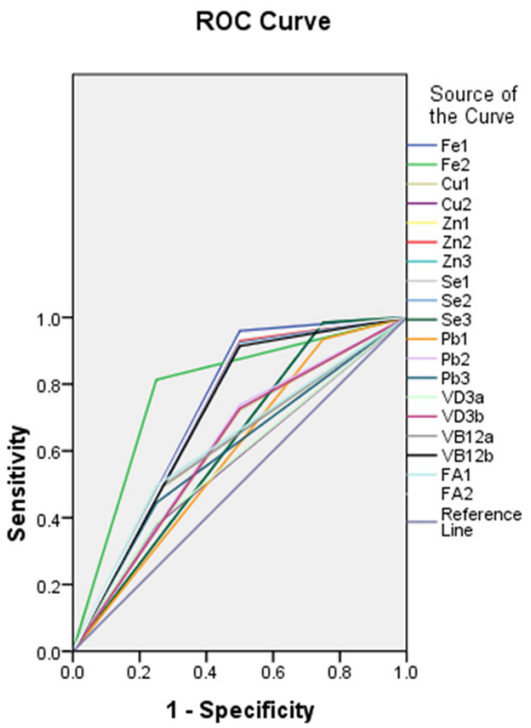

Figure 1. ROC curves of Fe, Cu, Zn, Se, Pb, vitamin D_3, vitamin B_{12} and FA applied in predicting CAD. Fe1: ROC curve for the serum level of Fe>1.71 mg/L applied in predicting CAD (AUC=0.730, SE=0.020, *P*<0.001), Fe2: ROC curve for the serum level of Fe>2.80 mg/L (AUC=0.782, SE=0.020, *P*<0.001); Cu1: ROC curve for the serum level of Cu>0.67 mg/L (AUC=0.611, SE=0.024, *P*<0.001), Cu2: ROC curve for the serum level of Cu>0.92 mg/L (AUC=0.564, SE=0.025, *P*=0.010); Zn1: ROC curve for the serum level of Zn≤0.87 mg/L (AUC=0.615, SE=0.025, *P*<0.001), Zn2: ROC curve for the serum level of Zn≤1.08 mg/L (AUC=0.715, SE=0.021, *P*<0.001), Zn3: ROC curve for the serum level of Zn≤1.53 mg/L (AUC=0.617, SE=0.023, *P*<0.001); Se1: ROC curve for the serum level of Se≤0.42 mg/L (AUC=0.617, SE=0.025, *P*<0.001), Se2: ROC curve for the serum level of Se≤0.67 mg/L (AUC=0.712, SE=0.021, *P*<0.001), Se3: ROC curve for the serum level of Se≤1.10 mg/L (AUC=0.617, SE=0.023, *P*<0.001); Pb1: ROC curve for the serum level of Pb>0.067 mg/L (AUC=0.592, SE=0.024, *P*<0.001), Pb2: ROC curve for the serum level of Pb>0.097 mg/L (AUC=0.619, SE=0.024, *P*<0.001), Pb3: ROC curve for the serum level of Pb>0.142 mg/L (AUC=0.597, SE=0.025, *P*<0.001); VD3a: ROC curve for the serum level of vitamin D_3≤29.67 μg/dL (AUC=0.567, SE=0.025, *P*<0.001), VD3b: ROC curve for the serum level of vitamin D_3≤36.47 μg/dL (AUC=0.614, SE=0.024, *P*<0.001); VB12a: ROC curve for the serum level of vitamin B_{12}≤49.57 μg/dL (AUC=0.615, SE=0.025, *P*<0.001), VB12b: ROC curve for the serum level of vitamin B_{12}≤68.41 μg/dL (AUC=0.707, SE=0.021, *P*<0.001); FA1: ROC curve for the serum level of FA≤10.28 μg/dL (AUC=0.622, SE=0.025, *P*<0.001), FA2: ROC curve for the serum level of FA≤13.57 μg/dL (AUC=0.722, SE=0.021, *P*<0.001); ROC: Receiver operating characteristic; CAD: Coronary artery disease; AUC: Area under curve.

excess Fe and Cu can generate deleterious free radicals which may stimulate the lipid peroxidation and eventually result in subsequent tissue damage. A study shows that elevated Fe concentration may generate reactive oxygen species (ROS) related with the promotion of atherogenesis and prothrombotic events, which may predispose to coronary disease [17]. As a toxic trace element for humans, Pb may competitively inhibit the absorption of important trace minerals, deactivate antioxidant sulphydryl pools and directly interrupt the activity of enzymes [18]. It induces free radical damage through affecting the formation of ROS and depleting the cellular antioxidant pool [19, 20]. A study shows that Pb exposure is positively associated with clinical CAD, cardiovascular and stroke mortality [21]. Moreover, an animal experiment shows that chronic exposure to Pb leads to arterial hypertension that persists long after ceasing Pb exposure [22]. Cebi A *et al*. found that the level of serum Pb was higher in CAD patients than in healthy controls [6]. In this study, elevated levels of serum Fe, Cu and Pb were independent risk factors of CAD.

Zn and Se are important trace elements for maintaining endothelial integrity because they can inhibit the events related with inflammation and oxidative stress [23, 24]. Contrary to redox-sensitive elements, Zn can act as an antioxidant agent. It may reduce the formation of free radicals and protect sulphydryl groups of proteins from the attack of free radicals [25, 26]. Furthermore, Zn has an important role in the activities of caspases, nitric oxide synthase and nuclear factor Kappa B (NF-κB) [27]. Se may also decrease the activity of NF-κB during inflammatory response in endothelium through interacting with this transcription factor, thereby helping to prevent atherosclerosis [28]. Reunanen A *et al*. found that a low serum Zn level had been correlated with increased cardiovascular mortality [11]. Renate Schnabel *et al.* observed that Se supplementation might enhance antioxidant capacity in vivo and in vitro for CAD patients [29]. In the present study, decreased levels of serum Zn and Se were independent risk factors of CAD.

Vitamin D insufficiency is strongly associated with endothelial dysfunction, subclinical atherosclerosis and slow coronary flow in patients with normal or near-normal coronary arteries at coronary angiography [30]. It has been reported that low vitamin D level is linked to higher

coronary artery calcium scores, increased vascular stiffness, increased mean platelet volume and inflammation [31, 32]. Studies show that vitamin D insufficiency is correlated with CAD and myocardial infarction [33] and is found in a high proportion for patients with myocardial infarction [34, 35]. Plenty of studies have shown that high level of serum homocysteine (Hcy), which is called hyperh-omocysteinemia (HHcy), has been associated with endothelial dysfunction of atherosclerotic CAD [36-42]. Vitamin B_{12} and FA have an important role in regulating the metabolic process of Hcy [43]. Recent studies have confirmed that the supplementation of vitamin B_{12} and FA may reduce the level of Hcy in patients with HHcy [44]. The supplementation of FA not only may reduce the level of Hcy in patients with HHcy [45], but also may significantly improve endothelial dysfunction in CAD patients [46]. On the contrary, vitamin B_{12} insufficiency and/or FA insufficiency may lead to HHcy [47-49]. In our study, decreased levels of vitamin D_3, vitamin B_{12} and FA were independent risk factors of CAD.

After independent risk factors were identified, their values in predicting CAD were evaluated using ROC curves. The results showed that the serum level of Fe>1.71 mg/L, Zn≤1.08 mg/L, Se≤0.67 mg/L, vitamin B_{12}≤68.41 μg/dL and FA≤13.57 μg/dL had moderate values (AUC> 0.700) when applied in predicting CAD. Therefore, the serum levels of Fe, Zn, Se, vitamin B_{12} and FA could be applied in predicting CAD.

In conclusion, elevated serum levels of Fe, Cu and Pb and decreased serum levels of Zn, Se, vitamin D_3, vitamin B_{12} and FA were independent risk factors of CAD. Moreover, the serum levels of Fe, Zn, Se, vitamin B_{12} and FA could be applied in predicting CAD.

Disclosure of conflict of interest

None.

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