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CLINICAL STUDIES

Serum copper and zinc concentrations are lower in Iranian patients with angiographically defined coronary artery disease than in subjects with a normal angiogram

Seyyed Mohammad Reza Kazemi-Bajestani[a], Majid Ghayour-Mobarhan[a,b,*], Mahmoud Ebrahimi[a,c], Mohsen Moohebati[a,c], Habib Allah Esmaeili[a,d], Mohammad Reza Parizadeh[a,b], Rasol Aghacizadeh[a,b], Gordon A.A. Ferns[e]

[a]*Atherosclerosis Research Center, Bu-Ali Research Institute, Mashhad University of Medical Science, Mashhad, Iran*
[b]*Department of Nutrition and Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Post Code: 9138813944, Mashhad, Iran*
[c]*Department of Cardiology, Faculty of Medicine, Mashhad University of Medical Sciences, Post Code: 9138813944, Mashhad, Iran*
[d]*Department of Statistics, Faculty of Medicine, Mashhad University of Medical Sciences, Post Code: 9138813944, Mashhad, Iran*
[e]*Centre for Clinical Science & Measurement, University of Surrey, Stag Hill, Guildford, Surrey GU2 7XH, UK*

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Abstract

Background: An imbalance between zinc and copper metabolism has been reported to predispose to coronary artery disease (CAD) in Western populations, but there are little data for other racial groups. We have therefore investigated the association between serum copper and zinc, and CAD in Iranian subjects undergoing coronary angiography.

Methods: Serum copper, zinc, fasting lipid profile, and blood glucose levels were measured in 114 patients (67 male and 47 female) undergoing routine coronary angiogram. Anthropometric features including blood pressure were determined using standard procedures. Demographic characteristics, including menopausal status and smoking habit, were assessed by questionnaire.

Results: Male patients had lower serum copper ($p<0.05$), lower serum zinc ($p<0.05$), and higher serum zinc/copper ratio ($p<0.05$) than females. Serum copper and zinc concentrations were significantly lower in the subjects with angiographically defined CAD than those patients with a normal angiogram, although the zinc/copper ratio was higher in these patients ($p<0.001$). Serum copper ($r=-0.303$, $p<0.001$) and zinc ($r=-0.250$, $p<0.01$) concentrations were both inversely related to age, and copper was positively associated with fasting serum triglycerides ($r=0.188$, $p<0.05$).

Conclusion: Serum copper and zinc concentrations were significantly lower in Iranian patients with abnormal versus those with a normal angiogram. However, the zinc/copper ratio was higher in patients with CAD compared to subjects without CAD. Serum zinc and copper concentrations appear to be influenced by several physiological factors including age and gender.
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Keywords: Copper; Zinc; Atherosclerosis; Angiography; Coronary artery disease

*Corresponding author. Atherosclerosis Research Center, Bu-Ali Research Institute, Mashhad University of Medical Science, Mashhad, Iran. Tel.: +98 9155171478; fax: +98 5118515119.
E-mail address: mjd-ghayour@mums.ac.ir (M. Ghayour-Mobarhan).

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Introduction

Several studies have demonstrated a relationship between copper status and cardiovascular disease in Western populations [1,2], but few investigations have been undertaken in other racial groups, or have examined this relationship in patients undergoing angiography.

The reported association between copper status and atherosclerosis has been inconsistent. Some studies have reported an association between copper deficiency and hypercholesterolemia, arterial lipid peroxide accumulation and endothelial dysfunction [1,3]. However, studies have also reported elevated levels of copper in the patients with coronary artery disease (CAD) [2,4]. Copper is a very effective catalyst of low-density lipoprotein (LDL) oxidation in vitro, which appears to play a pivotal role in atherogenesis [5]. Furthermore, copper activates several cholesterogenic genes in macrophages, which may provide another mechanism for the association between copper and atherosclerosis [6].

Low serum zinc concentrations are associated with an increased prevalence of CAD and diabetes and several associated risk factors including hypertension, hypertriglyceridemia, and other features associated with mild insulin resistance [7,8].

Klevay, in his Copper–Zinc Hypothesis, has suggested that an imbalance in the metabolism of zinc and copper predisposes to CAD. According to this hypothesis, a diet deficient in copper, either absolute, or relative to zinc intake, leads to hypercholesterolemia and an increased susceptibility to atherosclerosis in humans [9]. This hypothesis is supported by data from the studies of Singh et al. [10] and Bialkowska et al. [11].

The aim of this present study was to assess whether the hypothesis is applicable to other racial and geographical groups, using patients undergoing coronary angiography with or without evident disease.

Materials and methods

Subjects

The study was carried out on a sequential sample of 114 patients (67 males and 47 females) who were undergoing routine coronary angiography, mainly for stable angina, and who were positive for at least one objective test of myocardial ischemia including Exercise stress test, Dobutamin stress echocardiography, and Thallium spect. These tests and the angiogram were performed at the Ghaem Medical Education Hospital, Mashhad, Iran.

Patients ranged in age from 34 to 80 years. Patients who were on lipid-lowering medication, oral contraceptives, or hormone replacement therapy were excluded from the study. None of our subjects had a prior history of coronary angioplasty or coronary artery bypass graft (CABG), and pregnant women were excluded from the study.

However, 59 (51.7%) subjects were taking anti-hypertensive medication and 20 (17.5%) of the subjects used anti-diabetic drugs.

None of the subjects had overt clinical features of infection, or chronic inflammatory disease, and all subjects were negative for HBS antigen, anti-HCV antibody, and anti-HIV antibody.

Data about smoking habit and menopausal status were collected from each subject.

Each patient gave informed written consent to participate in the study, which was approved by the Mashhad University of Medical Science Ethics Committee.

Anthropometric measurement

For all patients, anthropometric parameters including weight, height, and waist circumference were measured using standard protocols. Waist circumference was measured at the level of the umbilicus (at the level midway between the lower rib margin and the iliac crest). Height, body weight, and waist circumference were measured with subjects dressed in very light clothing after an overnight fast. Body weight of each subject was measured with a standard scale to an accuracy of ± 0.1 kg, and height was measured to an accuracy of ± 0.1 cm. Blood pressure was measured twice while patients were seated, and rested for 15 min, using a standard mercury sphygmomanometer calibrated by Iranian Institute of Standards and Industrial Research. The interval between each blood pressure measurement was at least 30 min, and the average of the two measurements was taken as the blood pressure. The systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1) and the diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflating the cuff. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Angiographic assessment

Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed offline by a Specialist Cardiologist. The presence of one or more stenoses $\geqslant 50\%$ in diameter of at least one major coronary artery (Left main, Right coronary artery, Left anterior descending, Circumflex) was considered evidence of significant CAD [CAD (+)] [12]. Patients in whom stenoses $\geqslant 50\%$ in diameter were not identified were considered to have a normal angiogram [CAD (−)].

Collection of serum samples

Blood samples were collected in the morning from each subject after an overnight fast. After being allowed to clot, blood was then centrifuged at 2500 rpm for 15 min at room temperature to obtain serum. Hemolyzed samples were excluded from analysis. Serum was stored at −20 °C prior to analysis. All glassware and bottles used for the storage of serum and for analysis were previously soaked in diluted nitric acid (10%) for 3 h and rinsed thoroughly with de-ionized water in order to reduce the possibility of contamination with zinc or copper.

Routine biochemical analysis

A full fasted lipid profile, comprising total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), was determined for each patient. Serum lipid and fasting blood sugar glucose concentrations were measured by enzymatic methods.

Serum zinc and copper analysis

Serum copper and zinc concentrations were measured by flame atomic absorption spectrometry following a one in four dilution with distilled water [13]. Typical between-batch precision coefficients of variations [CVs] for these assays were 3.9% and 2.7%, respectively.

Statistical analysis

The data were subjected to statistical evaluation, using MiniTab (release 13, Minitab Inc, 2000, USA), with descriptive statistics (mean, median, standard deviation [SD], and interquartile range) being determined for all variables. Data were assessed for normality using the Kolomogorov–Smirnov test. In our comparisons *t*-tests and chi-square tests were used for quantitative and qualitative variables using a Bonferoni correction for multiple comparisons. Correlations were assessed using Pearson correlation coefficients. A $p<0.05$ was considered significant. Analysis of covariance (ANCOVA) was used to assess differences after adjustment for important confounding factors include age, gender, smoking habit, blood pressure, fasting blood glucose, lipid profile, BMI, and menopausal status. A person who had fasting blood glucose $\geqslant$126 mg/dL [14] or who was prescribed anti-diabetic medication was considered to be diabetic.

Results

Characteristics of the sample group

The mean age of subjects was 56.0 ± 11.0 years of whom 67 (58.8%) were male and 47 (41.2%) were female.

Of these patients 81 (71.1%) had angiographically defined CAD [CAD (+)] and 33 (28.9%) subjects had a normal angiogram [CAD (−)]. Among the 81 subjects who were CAD (+), 47 (58%) were male and 34 (42%) were female, and of those 33 who were CAD (−), 20 (60.7%) were male and 13 (39.3%) were female.

The mean serum copper and zinc concentrations in the whole patient group was 17.2 ± 0.38 μmol/L (range 9.42–26.69 μmol/L) and 12.36 ± 0.18 μmol/L (range 9.07–17.73 μmol/L), respectively, and the mean serum Zn/Cu ratio for the group was 0.83 ± 0.16.

The mean values for serum copper and zinc concentrations were significantly higher in females compared to males in the group as a whole ($p<0.05$, Table 1), and the mean value of the Zn/Cu ratio was significantly higher in males compared to females ($p = 0.015$, Table 1).

Smoking and physiological variations and trace elements

The mean values of serum copper and zinc concentration and zinc/copper ratio did not differ between smokers and non-smokers ($p>0.05$, Table 1).

The mean values of serum copper concentration, serum zinc concentration, and zinc/copper ratio were not significantly different between pre-menopausal and post-menopausal females ($p>0.05$, Table 1).

The mean values of serum copper concentration, serum zinc concentration, and zinc/copper ratio were not statistically different between obese (BMI > 30) and non-obese patients ($p>0.05$, Table 1).

Age, coronary risk factors and trace elements

Serum copper concentrations were positively associated with serum triglycerides ($p = 0.045$) and negatively associated with age ($p = 0.001$, Table 3). The serum zinc concentration was negatively associated with age ($p = 0.007$, Table 3).

There were no significant correlation between the other coronary risk factors and serum concentrations of copper and zinc, nor the zinc/copper ratio ($p>0.05$, Table 3).

Comparisons of metabolic risk factors in CAD (+) and CAD (−) groups

The CAD (+) group was approximately 6 years older than the CAD (−) group ($p = 0.005$). The male/female

Table 1. Relationship between gender, obesity, smoking and menopausal status on serum zinc and copper concentrations

	n (%)	Copper (μmol/L)	Zinc (μmo/L)	Zn/Cu
Gender				
Male	67 (58.7)	16.4 ± 0.36*	11.7 ± 0.18*	0.91 ± 0.17*
Female	47 (41.3)	18.3 ± 0.40	13.3 ± 0.19	0.72 ± 0.15
Smoking				
Yes	28 (24.6)	16.1 ± 0.33	11.5 ± 0.16	0.71 ± 0.15
No	86 (75.4)	17.5 ± 0.400	11.8 ± 0.38	0.67 ± 0.18
Menopause (in females)				
Yes	26 (55.3)	17.5 ± 0.39	11.7 ± 0.22	0.66 ± 0.15
No	21 (44.7)	19.1 ± 0.39	11.8 ± 0.16	0.61 ± 0.14
Obesity (BMI > 30)				
Yes	21 (18.4)	18.1 ± 0.39	12.2 ± 0.15	0.67 ± 0.15
No	93 (81.6)	16.9 ± 0.38	11.5 ± 0.185	0.68 ± 0.16

Values are expressed as mean ± SD. Between groups comparisons were assessed by *t*-test. BMI = body mass index; Zn = zinc; Cu = copper.
*$p<0.05$.

ratio, and proportion of current smokers did not differ between the CAD (−) and CAD (+) groups ($p>0.05$). The number of post-menopausal women were significantly higher in the CAD (+) group compared to the CAD (−) group ($p<0.05$). Indices of adiposity were higher in the CAD (+) group, with waist circumference ($p<0.001$) and BMI ($p<0.05$) being significantly higher than for the CAD (−) group, although the proportion with obesity (BMI > 30 kg/m^2) did not differ between the groups.

Systolic and diastolic blood pressures were not significantly different between CAD (−) and CAD (+) group ($p>0.05$ Table 2).

Fasting blood glucose, TG, LDL, and total cholesterol concentrations were significantly higher and HDL cholesterol lower for the CAD (+) group compared to the CAD (−) group ($p = 0.001$).

The proportion of diabetics in the CAD (+) and CAD (−) groups did not differ significantly ($p>0.05$). Statistical comparison of the proportion of subjects taking either anti-diabetic, or anti-hypertensive medication did not differ between the two groups ($p>0.05$, Table 2).

Variation of trace elements in CAD (−) and CAD (+) groups

The mean value of serum copper in the CAD (−) group was significantly higher than the CAD (+) group ($p = 0.001$, Table 2). The mean value of serum zinc concentration was also significantly higher in the CAD (−) than the CAD (+) group ($p = 0.001$, Table 2), and the mean value of Zn/Cu ratio was significantly higher in the CAD (+) group than the CAD (−) group ($p = 0.001$, Table 2).

These differences between CAD (−) and CAD (+) groups remain significant after adjusting for several confounding factors that included age, menopausal status, waist circumference, triglyceride, HDL, LDL, and total cholesterol.

Discussion

Association between trace element status and CAD in Iranian subjects

Despite the lack of accurate mortality data there is enough evidence to indicate that CAD is increasing in prevalence in Iran [15], as elsewhere in the Middle East. The mean serum values of trace elements were lower in our subjects than has been reported by others for Middle Eastern populations, although of a similar order [16], and may be related to the characteristics of the population sample, that is they were patients under investigation for chest pain, or related to dietary and other lifestyle factors.

Serum zinc and copper concentrations were lower in Iranian subjects with angiographically defined CAD, compared to patients without CAD. However, the serum zinc/copper ratio was found to be higher in the CAD (+) group than in the CAD (−) group.

It has been reported that copper deficiency is associated with impaired endothelium-dependent arterial relaxation that may result in coronary susceptibility [3]. Nevertheless, there are some studies that have reported a positive relationship between serum copper levels and atherosclerosis [2,4,17], and Kuliczkowski et al. [1] found that serum copper levels were in the normal range in patients with ischemic heart disease. Hence the relationship appears to be an inconsistent

Table 2. Correlation (*r*) between indices of serum trace elements status and individual coronary risk factors

	Copper (μmol/L)	Zinc (μmol/L)	Zn/Cu
Age	−0.303***	−0.250**	0.120
FBS (mg/dL)	0.113	0.171	−0.017
Triglyceride (mg/dL)	0.188*	0.062	0.100
HDL (mmol/L)	0.172	0.055	−0.121
LDL (mmo/L)	0.064	0.014	−0.070
Total cholesterol (mmol/L)	0.103	0.056	−0.131
Waist circumference (cm)	0.048	0.120	−0.131
BMI (kg/m^2)	0.055	0.111	0.035
Systolic blood pressure (mm/Hg)	−0.093	−0.017	0.058
Diastolic blood pressure (mm/Hg)	−0.024	0.092	0.051

Correlations were assessed using Pearson correlation coefficients. Non-normally distributed data such as triglycerides log transformed before using the Pearson correlations.
FBS = Fasting blood sugar; HDL = high-density lipoprotein; LDL = low-density lipoprotein; BMI = body mass index.
*$p<0.05$.
**$p<0.01$.
***$p<0.001$.

one, which may be affected by geographic location and racial background.

Zinc deficiency may also predispose to CHD [7,8]. As zinc is essential for several key enzymes and plays a role in the biosynthesis and storage of insulin in the beta-cells [18], zinc deficiency has been proposed to predispose to glucose intolerance, diabetes mellitus, insulin resistance, and atherosclerosis [8]. However, we found no relationship between fasting blood glucose and zinc status in our population sample.

Klevay proposed that an imbalance in zinc/copper metabolism predisposes to CAD [9,10]. According to this hypothesis, dietary copper deficiency, either alone or in association with a high zinc intake, may lead to hypercholesterolemia and the development of atherosclerosis [9].

This present study does not elucidate whether the lower levels of serum zinc and copper in the CAD (+) patients, is causal or the consequence of CAD; however, it is consistent with Klevay's hypothesis. Prospective studies would be necessary to confirm a causal relationship.

Gender and trace element status

The mean serum copper concentration was significantly higher in females, which is in accordance with the findings of several other reports [19,20]. These findings may be due to differences in diet between males and females as well as the reported differences in copper absorption between the genders [17].

Table 3. Clinical and biochemical characteristics of patients with and without CAD

	CAD (−) *n* = 33	CAD (+) *n* = 81
Serum copper (μmol/L)	18.1±0.36	16.8±0.39***
Serum zinc (μmol/L)	13.6±0.20	11.6±0.18***
Serum Zn/Cu ratio	0.61±0.13	0.74±1.75***
Age (years)	51.7±9.4	57.8±11.2**
Male/female	20/13	47/34
Smokers (%)	18.1%	27.1%
Menopausal (% of females)	30.7%	64.7%*
Waist circumference (cm)	88.4±12.0	95.4±10.1***
BMI (kg/m^2)	25.1±5.1	26.4±5.8*
Systolic BP (mmHg)	129.2±19.0	130.0±18.2
Diastolic BP (mmHg)	81.7±13.6	83.2±10.6
Fasting blood glucose (mmol/L)	6.2±2.8	6.6±4.9***
Triglyceride (mmol/L)	1.28 (1.03–1.39)	1.6 (1.12–1.81)***
HDL cholesterol (mmol/L)	1.31±0.42	1.14±0.26***
LDL (mmol/L)	2.93±0.91	3.51±1.4***
Total cholesterol (mmol/L)	4.47±1.22	4.92±1.14***
Obesity [BMI > 30] (%)	18.1%	18.5%
DM (%)	30.3%	27.2%
Anti-DM medication	21.2%	16.1%
Anti-hypertension medication	51.5%	51.9%

Values expressed as mean±SD for normally distributed data and median and interquartile range for not normally distributed data; between groups comparisons were assessed by *t*-test or chi-square test. Non-normal distribution data (serum triglycerides) were logarithmically transformed prior to parametric analysis. Zn = Zinc; Cu = copper; BMI = body mass index; FBS = fasting blood sugar; HDL = high-density lipoprotein; LDL = low-density lipoprotein; DM = diabetes mellitus; CAD = coronary artery disease.
*$p<0.05$.
**$p<0.01$.
***$p<0.001$.

The mean value of serum zinc concentrations in females was significantly higher than for males. Schuhmacher et al. [21] have reported similar results but our previous study has reported higher zinc concentration in males [20].

The mean value of Zn/Cu ratio was significantly higher in the male group, similar to our previous finding in a Caucasian population from the UK [20].

Effects of age and the menopause on trace element status

We found an inverse relationship between age and serum copper concentration in our sample population. These findings are similar to previous reports [19,20].

It has been suggested that these age-related changes of copper status may be due to altered absorption or excretion of copper [19].

We also found a consistent change in serum zinc concentration with age in our subjects. A fall in plasma zinc with age has been reported and attributed to a decline in rate of absorption and/or an accelerated clearance from the plasma [22]. There have also been other reports of changes in zinc metabolism with age [23].

Menopausal status may further complicate interpretation of trace element status because serum copper is affected by estrogen status [19,22,23]. None of our subjects were on hormone replacement therapy or the contraceptive pill. Serum copper and zinc concentrations and also the zinc/copper ratio were not significantly different between pre-menopausal and post-menopausal females. This is in contrast to the report of Di Gioacchino et al. [24] in their group of Italian post-menopausal women. However, the interpretation of the data in our study may be less problematic because none of women in our study used HRT or contraceptives.

Effects of smoking on trace element status

In the current study, we found no significant difference in serum concentrations of zinc, copper and zinc/copper ratio between smokers and non-smokers. We have previously reported that within a healthy Caucasian population, serum zinc and copper concentrations are reduced in smokers [20], however, this may be dependent on the type and form of tobacco consumption, which is likely to differ between the two populations.

Adiposity and trace element status

Mean serum zinc and copper concentrations were found to be non-significantly higher in obese Iranian subjects. We have previously found that serum copper concentrations were higher and the zinc/ copper ratio lower in obese dyslipidemic subjects, compared to non-obese dyslipidemic and healthy, non-obese controls in subjects from the UK (Ghayour-Mobarhan et al., unpublished data). Other investigators have reported that serum zinc concentrations are lower [20,25] and serum copper higher [20,25] in obese subjects, while other studies have reported no significant difference in serum zinc concentration between obese and non-obese subjects [26].

It is important to note that some of the subjects in our study had co-morbidities, including diabetes mellitus and CHD that may affect trace element status, in particular as CHD may be viewed as a chronic inflammatory condition, and this may have an impact on trace element status.

Trace elements and metabolic risk factors

We found a positive relationship between serum triglycerides and copper concentrations. There have been previous reports of a positive association between serum copper and triglycerides [27]. We found no other significant relationships between lipid-related parameters and trace element status, although there has been another report indicating an association between serum HDL and copper [20] status. We did not find a significant association between serum copper and LDL, as has been reported in other human studies [20,28]. Although high environmental exposure to copper may be associated with high serum LDL concentration [29], there have also been reports of an association between copper deficiency and hypercholesterolemia [30]. None of the patients in our study were being treated with lipid medication at the time of blood collection, but it is possible that treatment with anti-hypertensive or anti-diabetic medication may alter the relationship.

In conclusion, serum zinc and copper concentrations are significantly lower in Iranian patients with established CAD compared to patients with normal angiograms. The zinc/copper ratio was higher in patients with angiographically defined coronary disease. Demographic features such as gender and age are associated with altered zinc and copper status. It is unclear whether the lower serum zinc and copper concentration in patients with CAD might be causally related to atherosclerosis.

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