Comparison of serum levels of zinc, copper, and selenium between patients with coronary artery disease and healthy controls; Selenium could be a new diagnostic biomarker

Running title: The diagnostic value of trace elements in CAD

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Abstract

Background: Although several clinical and laboratory diagnostic approaches have been developed for coronary artery disease (CAD), rates of mortality and morbidity have remained high, which may be due to defective diagnostic markers. Here, we evaluated the serum levels of selected trace elements (Zn, Se, and Cu) in CAD patients and healthy subjects and assessed their diagnostic values in CAD.

Methods: 53 cardiologist-approved CAD patients and 48 age and sex-matched healthy controls were recruited. The serum levels of Zn, Se, and Cu were assessed using the atomic absorption method. GraphPad v.8.4 and SPSS v.18 software were used for statistical analyses.

Results: The serum levels of Zn and Se were significantly lower in the CAD patients than in controls ($P = 0.0008$ and $P < 0.0001$, respectively). In contrast, CAD patients showed significantly higher levels of Cu ($P = 0.0064$). Concerning the ROC curve analyses, the area under the curve (AUC) for Zn was 0.6563 ($P = 0.0069$). Setting the cut-off value at 1.255 µg/mL gave a sensitivity of 56.60%, specificity of 66.67%, and likelihood ratio (LR) of 1.698. AUC for Se was 0.8595 ($P<0.0001$). The optimum cut-off value at 86.50 ng/ml level gave a sensitivity of 83.02%, specificity of 75.00%, and LR of 3.321. The AUC for Cu was 0.6557 ($P = 0.0071$). The optimum cut-off value at 1.225 µg/mL level gave a sensitivity of 62.26%, specificity of 64.58%, and LR of 1.758.

Conclusion: Our study showed that selenium can be a biomarker with reliable diagnostic value for CAD.

Keywords: Trace elements, Coronary artery disease, Lipid profile, Diagnostic marker

Introduction

Coronary Artery Disease (CAD) is one of the most common types of cardiovascular disorders, which is caused by the association of various genetic and environmental risk factors (1). Multiple clinical and laboratory diagnostic methods have been developed for coronary artery disease, such as electrocardiograms, coronary calcium scans, CT coronary angiograms, coronary catheterizations, exercise stress tests, pharmacologic stress tests, and nuclear stress tests. However, the rates of mortality and morbidity associated with this condition remain high.(2). Since most diagnostic methods rely on examining clinical manifestations or introducing the disease's consequences (not predisposing factors) as diagnostic biomarkers, more powerful biomarkers are needed for the prognosis before the onset of illness (3).

Trace elements are essential minerals for various physiological functions in living organisms at low concentrations, which may possess toxic effects if consumed at high levels for long periods (4). They play imperative roles as cofactors for enzymes and other proteins and possess remarkable roles in vital chemical reactions (5). Various studies have revealed the association of trace elements levels and their distribution with immunological and inflammatory conditions (6). Moreover, the association of trace elements levels with the CAD has been highlighted in several studies (7, 8). Trace elements have been suggested as significant cardio-protective molecules in adequate pharmacologic concentrations (9). Some trace elements such as zinc (Zn), copper (Cu), and selenium (Se) could play remarkable roles in cardiovascular function and as the essential components of antioxidant enzymes to protecting cells against oxidative damage, have been altered in CAD (10). Although the role of these elements in the pathogenesis and predisposing CAD patients to develop the disease is well described, a few studies have evaluated their diagnostic value. Investigating the role of trace elements in the physiological and pathophysiological aspects of cardiovascular diseases can significantly enhance diagnosis, prognosis, and treatment strategies for affected patients.

Accordingly, we aimed to evaluate the serum levels of selected trace elements (Zn, Se, and Cu) in CAD patients and healthy subjects and assess their diagnostic values in distinguishing patients from controls.

Methods

Participants and sample collection

From January 2018 until September 2018, we enrolled 53 CAD patients (37 male, 16 female) from the hospitalized individuals in Modarres Hospital, Tehran, Iran. We also entered 48 age- and sex-matched normal controls (33 male, 15 female) from the healthy subjects referring to the Kowsar diagnostic laboratory, in Tehran. The study was performed under the guidelines of the Declaration of Helsinki (11). All participants were thoroughly informed about the study and signed a written consent form. CAD patients were examined at the Department of Cardiovascular Surgery at Modarres Hospital and included in the study after fulfilling the inclusion criteria. The lipid profile of all participants was also recorded. Coronary angiography was performed on all patients to confirm and evaluate the severity of CAD, following the European Association for Cardio-Thoracic Surgery (EACTS) (12) and the European Society of Cardiology (ESC) (13) guidelines. The Ethics Committee, Modarres Hospital, Tehran, Iran (IR.PII.REC.1395.98), had approved the study protocols and guidelines. CAD was diagnosed by an expert physician as remarkable left main coronary stenosis >50%, proximal left anterior descending stenosis >50%, presence of multivessel stenosis (narrowing >50% in coronary arteries) and complex coronary lesions. All of the patients with a history of infectious diseases, cancer, or other known chronic diseases were excluded. Briefly, 5 mL of whole blood was taken from all participants. The sera separated by centrifugation at 3000 rpm for 10 min and stored at −20 °C until use.

Determinations of serum concentrations of trace elements

In order to evaluate the serum levels of Zn (Reported as ppm or µg/mL), Se (ppb or ng/mL) and Cu (ppm or µg/mL), the Smith–Hieftje 22 automatic atomic absorption spectrometer (Thermo Jarrell Ash–Baird, Franklin, Massachusetts, USA) was utilized. To calibrate the device, several reference samples with specific concentrations were provided. The spectrometer measured their absorbance, plotting graphs of absorbance versus concentration for each element. It then drew the best-fit line with minimal error based on the standard sample data. All of the samples for both groups of CAD patients and controls were tested in triplicates and quality assurance guidelines were implemented.

Statistical analysis

Statistical analyses were conducted using GraphPad (Prism 8 for Windows, Version 8.4) and SPSS software (Windows version 18.0, Chicago, USA). Independent samples T-test was used to compare quantitative values between two groups. To evaluate the diagnostic value of each trace element, receiver operating characteristic (ROC) curves were applied and to define the threshold, the area under the curve (AUC) was calculated. The significance level was set at under 0.05 (P-value < 0.05).

Results

Lipid profile and the serum concentration of Zn, Se, and Cu in CAD patients and controls

The two groups were similar in gender (p-value = 0.788) and age (p-value = 0.198). The average age of patients with CAD was 54.61 years (± 5.97), while the average age of healthy controls was 52.68 years (± 7.73). The lipid profiles of the patients and controls were compared. Only the cholesterol levels in the CAD patients were higher than in the controls. Table 1 summarizes the clinical and laboratory parameters compared between CAD patients and healthy controls.

Table 1. The comparison of clinical and laboratory parameters between CAD patients and control

Characteristics	CAD patients (N: 53)	Healthy control (N: 48)	P-value*
Cholesterol	174.49±4.51	144.62±6.48	0.045
HDL	34.16±1.01	49.62±1.55	0.33
LDL	87.2±4.28	99.77±2.88	0.32
Triglycerides	156.13±8.51	146.67±7.97	0.16

* A p-value less than 0.05 indicates statistical significance. Results are presented as mean ± SD. Cholesterol in the CAD group was significantly higher than in the control group.

We found that serum levels of Zn were lower in the CAD patients compared to the healthy controls (P = 0.0008) (Figure 1A). Similarly, serum levels of Se were significantly decreased among CAD patients (P < 0.0001) (Figure 1B). However, CAD patients demonstrated significant higher levels of Cu in comparison to the healthy counterparts (P = 0.0064) (Figure 1C).

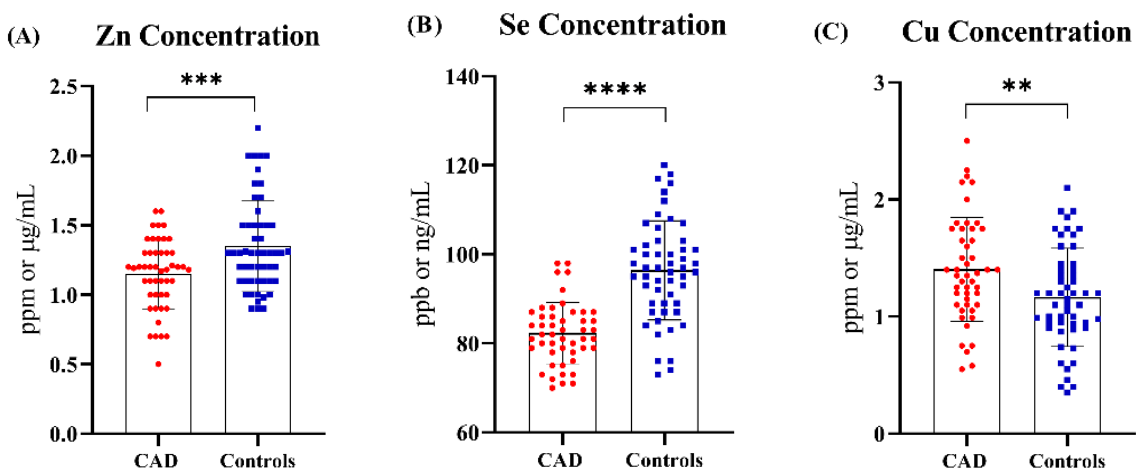
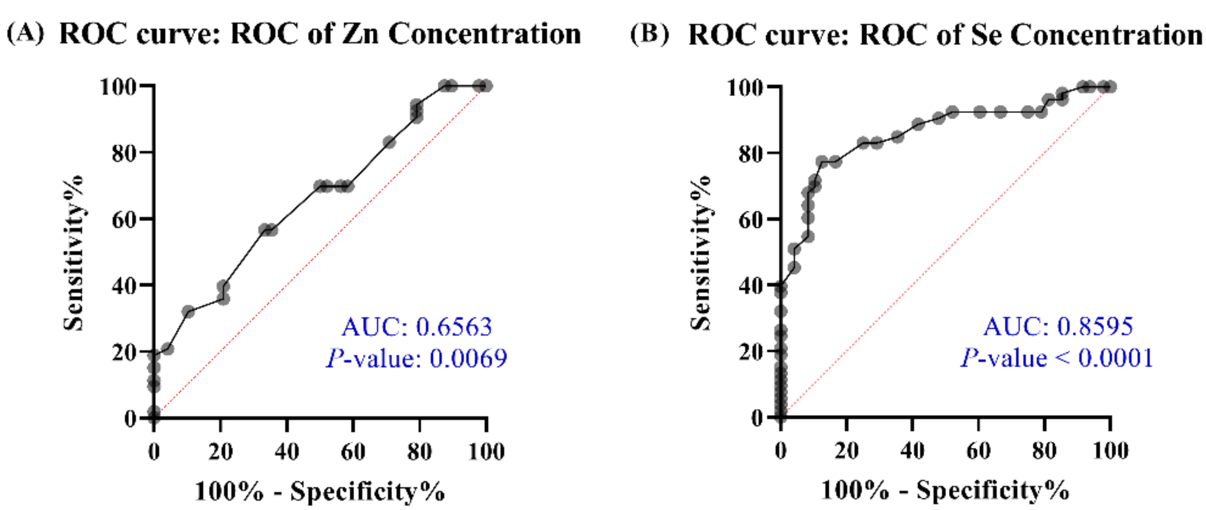


Figure 1. The serum concentration of Zinc (Zn), Selenium (Se), and Copper (Cu); Zn **(a)** and Se **(b)** serum levels are lower in CAD patients**,** while Cu is expressed in higher quantities **(c).** To compare the means between two groups, an independent samples T-test was used (Patients: 53, Healthy subjects: 48). Statistics on each scattered plot demonstrates Mean ± SD. Level of significant *P*-values were 0.05. **P-value<0.01, ***P-value<0.001, and ****P-value<0.0001

Selenium could be a suitable diagnostic marker for CAD

ROC curve analysis was performed on the serum levels of trace elements among CAD patients, to assess their diagnostic utility. The AUC for the serum levels of Zn was 0.6563 (95% CI: 0.5509-0.7616; P = 0.0069) which is worthless. The cut-off point was set at the level of 1.255 µg/mL, with a sensitivity of 56.60% (95% CI: 43.27-69.05), a specificity of 66.67% (95% CI: 52.54-78.32), and a likelihood ratio (LR) of 1.698 (Figure 2A). The calculated AUC for Se was 0.8595 (95% CI: 0.7854-0.9335; P<0.0001) which is excellent. The cut-off value was set at the level of 86.50 ng/ml with a sensitivity of 83.02% (95% CI: 70.77-90.80), a specificity of 75.00% (95% CI: 61.22-85.08), and a likelihood ratio (LR) of 3.321 (Figure 2B). The AUC for the serum levels of Cu was 0.6557 (95% CI: 0.5493-0.7620; P = 0.0071) which is worthless. The cut-off point was set at the level of 1.225 µg/mL, with a sensitivity of 62.26% (95% CI: 48.81-74.06), a specificity of 64.58% (95% CI: 50.44-76.57), and a likelihood ratio (LR) of 1.758 (Figure 2C).



(C) ROC curve: ROC of Cu Concentration

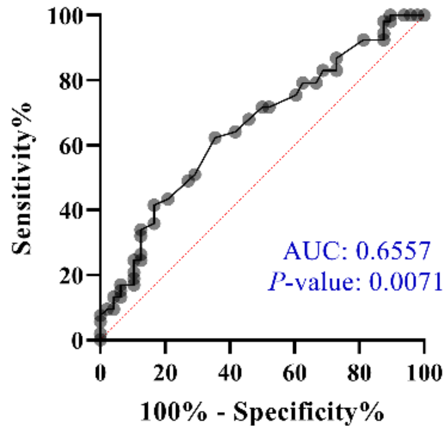


Figure 2. ROC curve analyses; Area under the curve (AUC) for Zn was 0.6563 (P-value = 0.0069). Setting the cut-off value at 1.255 µg/mL gave a sensitivity of 56.60%, specificity of 66.67% and likelihood ratio (LR) of 1.698 **(a)**. AUC for Se was 0.8595 (P-value<0.0001). The optimum cut-off value at the level of 86.50 ng/ml gave a sensitivity of 83.02%, specificity of 75.00% and likelihood ratio (LR) of 3.321 **(b)**. AUC for Cu was 0.6557 (P-value = 0.0071). The optimum cut-off value at the level of 1.225 µg/mL gave a sensitivity of 62.26%, specificity of 64.58% and likelihood ratio (LR) of 1.758 **(c)**.

Discussion

Even with significant progress in diagnosis and treatment, CAD remains one of the most life-threatening cardiovascular conditions globally, leading to high rates of mortality and morbidity (14). Although several genetic and environmental predisposing factors have been suggested, the levels of trace elements in the CAD patients, because of their important biological functions and heart-protective benefits, are of great importance (7). Recent studies demonstrated that antioxidant agents have an important role to prevent cardiovascular diseases. Accordingly, we evaluated the serum levels of selected trace elements (Zn, Se, and Cu) in CAD patients and healthy subjects and assessed their diagnostic values in distinguishing patients from controls.

We showed that serum levels of Zn were lower in the CAD patients compared to the healthy controls. Similar to other mentioned trace elements, Zn appears to possess protective effects in CAD by playing a crucial role in the redox-signaling pathway. In accordance with our findings, the decreased levels of Zn have been reported in various publications (15-17). Kazemi-Bajestani et al. demonstrated that people with abnormal angiograms have lower levels of Zn than those with normal angiograms (15). In Kosar et al. study, Zn serum levels were significantly lower in both CAD and coronary artery ectasia than in healthy controls. However, they did not approve of a relationship between Se and CAD or coronary artery ectasia (16). Similar to Zn, serum levels of Se were significantly reduced among CAD patients in our study. Due to the antioxidant properties of Se, it has been suggested that Se could prevent cardiovascular and other chronic inflammatory diseases. Moreover, Se supplementation may elevate the enzymatic activity of antioxidants and decrease lipid peroxidation, in favor of reducing the risk of CAD (18-20). Moreover, CAD patients demonstrated significantly higher levels of Cu compared to the healthy counterparts. Previous studies have revealed that CAD patients may have aberrantly altered levels of Cu and ineffective activities of some Cu-dependent enzymes. Consistent with our results, Ilyas and Shah showed that the measure of Cu was moderately increased in CAD patients compared to healthy people (21). An in-vivo experiment by Pan et al. demonstrated how long-term increased serum Cu levels can damage cardiomyocytes. This damage occurs through the deposition of copper on the extracellular matrix of cardiomyocytes and the increase of cytochrome C in the cytoplasm following the destruction of the mitochondrial outer membrane of these cells, which triggers cell apoptosis. Ultimately, these processes lead to the death of cardiomyocytes (22).

Despite significant differences in the levels of studied trace elements in the sera of CAD patients and healthy controls, a few studies have evaluated the diagnostic value of these molecules as putative diagnostic markers. The ROC curve analysis in our study showed that Se (AUC: 0.8595; $P < 0.0001$) could be introduced as a reliable diagnostic biomarker. The cut-off value for Se was set at the level of 86.50 ng/ml with a sensitivity of 83.02%, a specificity of 75.00%, and a likelihood ratio (LR) of 3.321. To the best of our knowledge, no study has proposed Se as a diagnostic biomarker for CAD. However, some studies have suggested Se as a biomarker for diagnosing neonatal infections, malignancies, and pulmonary arterial hypertension (23-25). Moreover, Zn (AUC: 0.6563; $P = 0.0069$) and Cu (AUC: 0.6557; $P = 0.0071$) were also regarded as acceptable diagnostic biomarkers. A recent publication by Knez et al. has suggested Zn as a powerful diagnostic marker for cardiovascular health which is in accordance with our findings (26). Moreover, Zn deficiency has been associated with the altered prognosis of cardiovascular diseases (27). Similar to what discussed about Se, no study has suggested Cu as a diagnostic marker for CAD, but proposing it as a biomarker in neonatal infection and neurodegenerative disorders (28). Although our research proposed Se as a novel diagnostic biomarker for CAD, it was accompanied by limitations, including small sample size, not evaluating the markers in different subgroups, and lack of follow-up, which should be compensated in more sophisticated further studies. Another limitation of this study was that none of the patients or controls were screened for diet and mineral supplement intake, which could have affected the results.

Conclusion

In our study, we observed lower serum levels of selenium and zinc, while copper levels were elevated in patients with CAD. Selenium emerged as a promising diagnostic biomarker for CAD, while zinc and copper were identified as useful markers for diagnosis. However, further research with larger sample sizes is essential to assess the diagnostic and prognostic value of these markers in differentiating CAD patients from healthy individuals and related groups. However, more sophisticated research with a bigger sample size would be necessary to evaluate these markers' diagnostic and prognostic value in distinguishing CAD patients from healthy subjects and related subgroups.

Acknowledgments

We would like to appreciate the scientific and technical support from all clinical and laboratory staff at Modarres Hospital, Tehran, Iran, and all of the participants (either patients or normal subjects) who donated their biological samples to this project.

Ethical Statements

The ethics committee at Payame Noor University approved this research project in advance (Code of Ethics: IR.PII.REC.1395.98).

Funding Source

This article is derived from a M.Sc. thesis in Biochemistry that was financially supported by Payame Noor University, Tehran Branch (Research code: 614).

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Study conception or design: R.S.; Data Processing, Collection, Perform Experiment: M.H.V. and M.E. and M.B.M. Preparation of clinical Samples: M.E.; Clinical consultants: R.S. and S.F.S.; Supervision of the research: R.S.; Manuscript preparation: M.H.V.;
Final version of the manuscript approved by all authors.

Data Availability Statement

The dataset presented in this study is available upon request from the corresponding author, either during submission or after publication. The data are not publicly available because they contain information that could compromise the privacy of research participants.

References

1. Ghassibe-Sabbagh M, Platt DE, Youhanna S, Abchee AB, Stewart K, Badro DA, et al. Genetic and environmental influences on total plasma homocysteine and its role in coronary artery disease risk. Atherosclerosis. 2012;222(1):180-6.
2. Cassar A, Holmes Jr DR, Rihal CS, Gersh BJ, editors. Chronic coronary artery disease: diagnosis and management. Mayo Clinic Proceedings; 2009: Elsevier.
3. Castiglione V, Aimo A, Vergaro G, Saccaro L, Passino C, Emdin M. Biomarkers for the diagnosis and management of heart failure. Heart Failure Reviews. 2021:1-19.
4. Rovira J, Hernández-Aguilera A, Luciano-Mateo F, Cabré N, Baiges-Gaya G, Nadal M, et al. Trace elements and paraoxonase-1 activity in lower extremity artery disease. Biological trace element research. 2018;186(1):74-84.
5. Bhattacharya PT, Misra SR, Hussain M. Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica. 2016;2016.
6. Mehri A. Trace elements in human nutrition (ii)–an update. International Journal of Preventive Medicine. 2020;11.
7. Cebi A, Kaya Y, Gungor H, Demir H, Yoruk IH, Soylemez N, et al. Trace elements, heavy metals and vitamin levels in patients with coronary artery disease. International Journal of Medical Sciences. 2011;8(6):456.
8. Noshin TF, Ali MR, Banik S. Increased oxidative stress and altered serum macro-minerals and trace elements levels are associated with coronary artery disease. Journal of Trace Elements in Medicine and Biology. 2021;64:126707.
9. Ekpenyong CE. Essential trace element and mineral deficiencies and cardiovascular diseases: facts and controversies. Int J Food Sci Nutr. 2017;6:53-64.
10. Ghayour-Mobarhan M, Taylor A, Kazemi-Bajestani S, Lanham-New S, Lamb DJ, Vaidya N, et al. Serum zinc and copper status in dyslipidaemic patients with and without established coronary artery disease. Clinical laboratory. 2008;54(9-10):321-9.

11. Goodyear MD, Krleza-Jeric K, Lemmens T. The declaration of Helsinki. British Medical Journal Publishing Group; 2007. p. 624-5.
12. Head SJ, Howell NJ, Osnabrugge RL, Bridgewater B, Keogh BE, Kinsman R, et al. The European association for cardio-thoracic surgery (EACTS) database: An introduction. European Journal of Cardio-Thoracic Surgery. 2013;44(3):e175-e80.
13. Association DwtscotEHR, Surgery EbtEAfC-T, Members ATF, Camm AJ, Kirchhof P, Lip GY, et al. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). European heart journal. 2010;31(19):2369-429.
14. Bauersachs R, Zeymer U, Brière J-B, Marre C, Bowrin K, Huelsebeck M. Burden of coronary artery disease and peripheral artery disease: a literature review. Cardiovascular therapeutics. 2019;2019.
15. Kazemi-Bajestani SMR, Ghayour-Mobarhan M, Ebrahimi M, Moohebati M, Esmaeili HA, Parizadeh MR, et al. Serum copper and zinc concentrations are lower in Iranian patients with angiographically defined coronary artery disease than in subjects with a normal angiogram. Journal of trace elements in medicine and biology. 2007;21(1):22-8.
16. Kosar F, Taskapan C, Kucukbay Z. Serum levels of selenium, zinc and copper in patients with coronary artery ectasia. Indian heart journal. 2007;59(1):38-41.
17. Little PJ, Bhattacharya R, Moreyra AE, Korichneva IL. Zinc and cardiovascular disease. Nutrition. 2010;26(11-12):1050-7.
18. Schnabel R, Lubos E, Messow CM, Sinning CR, Zeller T, Wild PS, et al. Selenium supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease: The SElenium Therapy in Coronary Artery disease Patients (SETCAP) Study. American heart journal. 2008;156(6):1201. e1-. e11.
19. Hassanzadeh M, Faridhosseini R, Mahini M, Faridhosseini F, Ranjbar A. Serum levels of TNF-a, IL-6, and selenium in patients with acute and chronic coronary artery disease. Iranian Journal of Immunology. 2006;3(3):142-5.
20. Seyyed Mohammad Reza P, Moohebati M, Ghafoori F, Ghayour-Mobarhan M, Kazemi-Bajestani SMR, Tavallaie S, et al. Serum selenium and glutathione peroxidase concentrations in Iranian patients with angiography-defined coronary artery disease. Angiology. 2009;60(2):186-91.
21. Ilyas A, Shah MH. Abnormalities of Selected Trace Elements in Patients with Coronary Artery Disease. Acta Cardiol Sin. 2015;31(6):518-27.
22. Pan M, Cheng Z-W, Huang C-G, Ye Z-Q, Sun L-J, Chen H, et al. Long-term exposure to copper induces mitochondria-mediated apoptosis in mouse hearts. Ecotoxicol Environ Saf. 2022;234:113329.
23. Hackler J, Wisniewska M, Greifenstein-Wiehe L, Minich WB, Cremer M, Bührer C, Schomburg L. Copper and selenium status as biomarkers of neonatal infections. Journal of Trace Elements in Medicine and Biology. 2020;58:126437.
24. Zhao Y, Sun Q, Zhang X, Baeyens J, Su H. Self-assembled selenium nanoparticles and their application in the rapid diagnostic detection of small cell lung cancer biomarkers. Soft Matter. 2018;14(4):481-9.
25. Sun Q, Hackler J, Hilger J, Gluschke H, Muric A, Simmons S, et al. Selenium and copper as biomarkers for pulmonary arterial hypertension in systemic sclerosis. Nutrients. 2020;12(6):1894.
26. Knez M, Glibetic M. Zinc as a Biomarker of Cardiovascular Health. Frontiers in nutrition. 2021;8.

27. Choi S, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. Acta Pharmacologica Sinica. 2018;39(7):1120-32.
28. Faber S, Zinn GM, Kern Ii JC, Skip Kingston H. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers. 2009;14(3):171-80.