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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

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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 remain high, possibly because of limitations in existing 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. 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: Serum levels of Zn and Se were significantly lower in CAD patients than in controls ($P = 0.0008$ and $P < 0.0001$, respectively). In contrast, CAD patients showed significantly higher Cu levels ($P = 0.0064$). Regarding 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 yielded a sensitivity of 56.60%, specificity of 66.67%, and likelihood ratio (LR) of 1.698. The AUC for Se was 0.8595 ($P<0.0001$). The optimum cut-off value of 86.50 ng/ml yielded 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 of 1.225 µg/mL yielded a sensitivity of 62.26%, specificity of 64.58%, and LR of 1.758.

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

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Coronary Artery Disease
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Diagnostic marker

Introduction

Coronary Artery Disease (CAD) is one of the most common cardiovascular disorders and is caused by the interaction of various genetic and environmental risk factors (1). Multiple clinical and laboratory diagnostic methods have been developed for coronary artery disease, including electrocardiograms, coronary calcium scans, CT coronary angiograms, coronary catheterizations, exercise stress tests, pharmacologic stress tests, and nuclear stress tests. However, mortality and morbidity rates associated with this condition remain high (2). Since most diagnostic methods rely on assessing clinical manifestations or using the consequences of the disease, rather than predisposing factors, as diagnostic biomarkers, more powerful biomarkers are needed for prognosis before disease onset (3).

Trace elements are essential minerals required at low concentrations for various physiological functions in living organisms; however, they may exert toxic effects when consumed at high levels for prolonged periods (4). They play essential roles as cofactors for enzymes and other proteins and have important functions in vital chemical reactions (5). Various studies have demonstrated associations between trace element levels and their distribution and immunological and inflammatory conditions (6). Moreover, associations between trace element levels and CAD have been highlighted in several studies (7,8). Trace elements have been suggested to act as important cardioprotective molecules at adequate pharmacologic concentrations (9). Some trace elements, such as zinc (Zn), copper (Cu), and selenium (Se), may play important roles in cardiovascular function and, as essential components of antioxidant enzymes that protect cells against oxidative damage, may be altered in CAD (10). Although the role of these elements in the pathogenesis of CAD and in predisposing patients to disease development is well described, 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 diagnostic, prognostic, 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 to 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 hospitalized individuals at Modarres Hospital, Tehran, Iran. We also included 48 age- and sex-matched normal controls (33 Male, 15 Female) from healthy subjects who were referred to the Kowsar diagnostic laboratory in Tehran. The study was performed in accordance with 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 were 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 of Modarres Hospital, Tehran, Iran (IR.PII.REC.1395.98), approved the study protocols and guidelines. CAD was diagnosed by an expert physician based on significant left main coronary stenosis >50%, proximal left anterior descending stenosis >50%, the presence of multivessel stenosis (Narrowing >50% in coronary arteries), and complex coronary lesions. All patients with a history of infectious diseases, cancer, or other known chronic diseases were excluded. Briefly, 5 mL of whole blood was collected from all participants. The sera were separated by centrifugation at 3000 rpm for 10 min and stored at -20 °C until use.

Determination of serum concentrations of trace elements

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 used. To calibrate the device, several reference samples with specific concentrations were prepared. The spectrometer measured their absorbance and generated plots of absorbance versus concentration for each element. It then generated the best-fit line with minimal error based on the standard sample data. All samples from both CAD patients and controls were tested in triplicate, 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 the two groups. To evaluate the diagnostic value of each trace element, receiver operating characteristic (ROC) curves were applied, and the area under the curve (AUC) was calculated to define the threshold. The significance level was set at under 0.05 (P-value < 0.05).

Results

Lipid profile and serum concentrations 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 cholesterol levels were higher in CAD patients than in controls. Table 1 summarizes the clinical and laboratory parameters compared between CAD patients and healthy controls.

Table 1. Comparison of clinical and laboratory parameters between CAD patients and controls

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 CAD patients than in 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 significantly higher levels of Cu compared with their healthy counterparts (P = 0.0064) (Figure 1C).

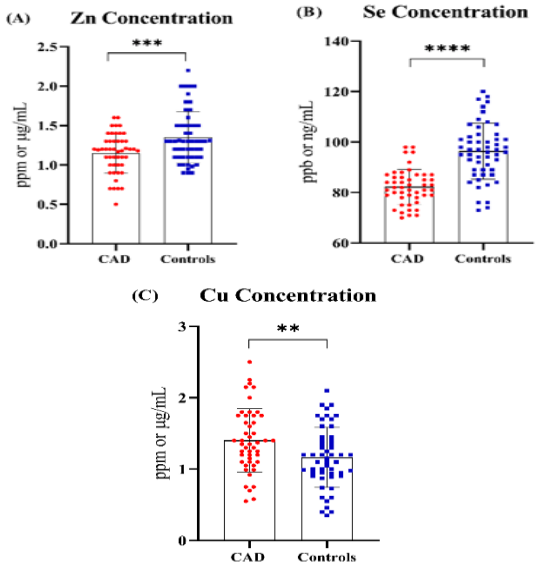

Figure 1. Serum concentrations of Zinc (Zn), Selenium (Se), and Copper (Cu); Zn (a) and Se (b) serum levels are lower in CAD patients, while Cu is expressed at higher levels (c). To compare the means between the two groups, an independent samples T-test was used (Patients: 53, Healthy subjects: 48). Statistics on each scatter plot demonstrate mean ± SD. The significance level for P-values was 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), indicating poor discriminatory ability. The cut-off point was set at 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), indicating excellent discriminatory ability. The cut-off value was set at 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), indicating poor discriminatory ability. The cut-off point was set at 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).

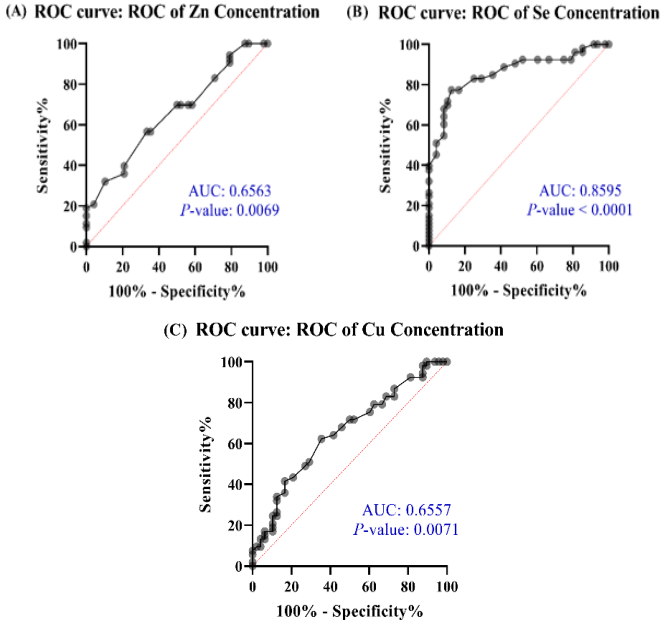

Figure 2. ROC curve analyses; the area under the curve (AUC) for Zn was 0.6563 (P-value = 0.0069). Setting the cut-off value at 1.255 µg/mL yielded a sensitivity of 56.60%, specificity of 66.67%, and likelihood ratio (LR) of 1.698 (a). The AUC for Se was 0.8595 (P-value<0.0001). The optimum cut-off value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

Discussion

Despite 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, trace element levels in CAD patients are of great importance because of their important biological functions and cardioprotective benefits (7). Recent studies have demonstrated that antioxidant agents play an important role in preventing 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 CAD patients than in healthy controls. Similar to the other trace elements mentioned, Zn appears to exert protective effects in CAD by playing a crucial role in the redox-signaling pathway. In accordance with our findings, 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 the study by Kosar et al., serum Zn levels were significantly lower in both CAD and coronary artery ectasia than in healthy controls. However, they did not confirm 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. Owing to the antioxidant properties of Se, it has been suggested that Se could prevent cardiovascular and other chronic inflammatory diseases. Moreover, Se supplementation may increase the enzymatic activity of antioxidants and decrease lipid peroxidation, thereby reducing the risk of CAD (18-20). Furthermore, CAD patients demonstrated significantly higher levels of Cu compared with their 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 Cu levels were moderately increased in CAD patients compared with 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 copper deposition on the extracellular matrix of cardiomyocytes and an increase in cytochrome C in the cytoplasm following destruction of the mitochondrial outer membrane of these cells, which triggers cell apoptosis. Ultimately, these processes lead to cardiomyocyte death (22).

Despite significant differences in the levels of the studied trace elements in the sera of CAD patients and healthy controls, 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 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. 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 altered prognosis in cardiovascular diseases (27). Similar to what was discussed for Se, no study has suggested Cu as a diagnostic marker for CAD; however, it has been proposed as a biomarker in neonatal infection and neurodegenerative disorders (28). Although our research proposed Se as a novel diagnostic biomarker for CAD, it had limitations, including a small sample size, lack of evaluation of markers in different subgroups, and lack of follow-up, which should be addressed in more sophisticated future studies. Another limitation of this study was that neither patients nor 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 diagnostic markers. 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.

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Ethical Statement

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

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Study conception or design: R.S.; Data processing, collection, and performance of experiments: M.H.V., 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.; The final version of the manuscript was 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.

Use of Artificial Intelligence

No artificial intelligence or large language model technologies were employed in the research, data analysis, or preparation of this manuscript.

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. [View at Publisher] [DOI] [PMID] [Google Scholar]
2. Cassar A, Holmes Jr DR, Rihal CS, Gersh BJ, editors. Chronic coronary artery disease: diagnosis and management. Mayo Clin Proc. 2009;84(12):1130-46. [View at Publisher] [DOI] [PMID] [Google Scholar]
3. Castiglione V, Aimo A, Vergaro G, Saccaro L, Passino C, Emdin M. Biomarkers for the diagnosis and management of heart failure. Heart Fail Rev. 2022;27(2):625-43. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. Biol Trace Elem Res. 2018;186(1):74-84. [View at Publisher] [DOI] [PMID] [Google Scholar]
5. Bhattacharya PT, Misra SR, Hussain M. Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica. 2016:2016:5464373. [View at Publisher] [DOI] [PMID] [Google Scholar]
6. Mehri A. Trace elements in human nutrition (ii)-an update. Int J Prev Med. 2020:11:2. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. Int J Med Sci. 2011;8(6):456-60. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. J Trace Elem Med Biol. 2021;64:126707. [View at Publisher] [DOI] [PMID] [Google Scholar]
9. Ekpenyong CE. Essential trace element and mineral deficiencies and cardiovascular diseases: facts and controversies. Int J Food Sci Nutr. 2017;6(2):53-64. [View at Publisher] [DOI]
10. Ghayour-Mobarhan M, Taylor A, Kazemi-Bajestani SMR, Lanham-New S, Lamb DJ, Vaidya N, et al. Serum zinc and copper status in dyslipidaemic patients with and without established coronary artery disease. Clin Lab. 2008;54(9-10):321-9. [View at Publisher] [DOI] [Google Scholar]
11. Goodyear MD, Krleza-Jeric K, Lemmens T. The declaration of Helsinki. BMJ. 2007;335(7621):624-5. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. Eur J Cardiothorac Surg. 2013;44(3):e175-e80. [View at Publisher] [DOI] [PMID] [Google Scholar]

13. Association EHR, Surgery EAfC-T, Camm AJ, Kirchhof P, Lip GY, Schotten U, 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). Eur Heart J. 2010;31(19):2369-429. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. J Trace Elem Med Biol. 2007;21(1):22-8. [View at Publisher] [DOI] [PMID] [Google Scholar]
16. Kosar F, Taskapan C, Kucukbay Z. Serum levels of selenium, zinc and copper in patients with coronary artery ectasia. Indian Heart J. 2007;59(1):3841. [View at Publisher] [PMID] [Google Scholar]
17. Little PJ, Bhattacharya R, Moreyra AE, Korichneva IL. Zinc and cardiovascular disease. Nutrition. 2010;26(11-12):1050-7. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. Am Heart J. 2008;156(6):1201.e1-.e11. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. Iran J Immunol. 2006;3(3):142-5. [View at Publisher] [DOI] [PMID] [Google Scholar]
20. Parizadeh SMR, 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. [View at Publisher] [DOI] [PMID] [Google Scholar]
21. Ilyas A, Shah MH. Abnormalities of Selected Trace Elements in Patients with Coronary Artery Disease. Acta Cardiol Sin. 2015;31(6):518-27. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. [View at Publisher] [DOI] [PMID] [Google Scholar]
23. Hackler J, Wisniewska M, Greifenstein-Wiehe L, Minich WB, Cremer M, Bührer C, et al. Copper and selenium status as biomarkers of neonatal infections. J Trace Elem Med Biol. 2020;58:126437. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. [View at Publisher] [DOI] [PMID] [Google Scholar]
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. [View at Publisher] [DOI] [PMID] [Google Scholar]
26. Knez M, Glibetic M. Zinc as a Biomarker of Cardiovascular Health. Front Nutr. 2021:8:686078. [View at Publisher] [DOI] [PMID] [Google Scholar]
27. Choi S, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. Acta Pharmacol Sin. 2018;39(7):1120-32. [View at Publisher] [DOI] [PMID] [Google Scholar]
28. Faber S, Zinn GM, Kern 2nd JC, Skip Kingston HM. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers. 2009;14(3):171-80. [View at Publisher] [DOI] [PMID] [Google Scholar]

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