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Comparative Analysis of Serum Levels of Calcium, Phosphorus, Magnesium, and Zinc in Individuals with Metabolic Syndrome versus Healthy Control

Saina Mirhosseini; MSc[1], Akram Ghadiri-Anari; MD [2*], Amin Moosavi; Medical student [3] & Mahnaz Zohal; Medical student [3]

[1] *Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran;* [2] *Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;* [3] *School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

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****Corresponding author:***
m_zohal69@yahoo.com
School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Postal code: 3415834937
Tel: +98 9127866320

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ABSTRACT

Background: Metabolic syndrome (MeTS) is directly associated with an increased risk of cardiovascular diseases and diabetes. The present study aimed to compare serum levels of calcium, phosphorus, magnesium, and zinc in the MeTS and control groups and determine the relationship between serum levels of calcium, phosphorus, magnesium, and zinc and components of MeTS. **Methods:** This case-control study was conducted on 149 individuals (79 people with MeTS and 70 healthy individuals). Demographic data, including age, gender, body mass index (BMI), waist circumference, and blood pressure, as well as laboratory data, including fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL-C), magnesium, calcium, phosphorus, and zinc, were collected. **Results:** The results indicated that serum zinc levels were significantly lower in MeTS group, while serum phosphorus, calcium, and magnesium levels did not show significant differences between the two groups. Furthermore, serum zinc levels significantly decreased as MeTS factors increased, particularly with 4 or 5 factors. Serum zinc levels were significantly associated with blood pressure, BMI, and HDL-C. Similarly, serum magnesium levels showed a significant association with total cholesterol. Serum calcium levels were linked to both HDL-C and BMI. In contrast, serum phosphorus levels showed no significant association with any components of MeTS. **Conclusion:** Decreased serum zinc levels appear to be significantly related to MeTS and its components, including blood pressure and HDL-C. However, the relationship between serum calcium, phosphorus, magnesium, and zinc levels and MeTS requires further studies.

Introduction

Metabolic syndrome (MeTS) refers to a cluster of endocrine and metabolic disorders that, when co-occurring, can pose a significant health risk to an individual. These disorders include high waist circumference, elevated fasting glucose levels, high triglyceride levels, decreased high-density lipoprotein cholesterol (HDL-C) levels, and hypertension. If at least three of these abnormalities are present, the individual is considered to have MeTS (IDF/NCEP criteria)

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(Fahed *et al.*, 2022).

Various factors, including insulin resistance, obesity, chronic inflammation, and genetic and acquired factors, play a role in the pathophysiology of MeTS, with insulin resistance appearing to have a particularly significant role. Due to its association with various diseases, particularly cardiovascular diseases (CVD) and diabetes, preventing, diagnosing, and treating MeTS are crucial. Ignoring it can lead to irreversible complications (Bovolini *et al.*, 2021, McCracken *et al.*, 2018). A study conducted in 2021 showed that MeTS patients, compared to those without MeTS, have a higher risk of all-cause mortality, cardiovascular death, myocardial infarction, and stroke. Additionally, MeTS increases the risk of cardiovascular-related complications in patients with CVD (Li *et al.*, 2021).

On the other hand, lifestyle factors such as nutrition, substance abuse, alcohol and tobacco use, physical activity, and sleep hygiene play an important role in MeTS. Nowadays, the use of appropriate dietary interventions in the management and treatment of MeTS has gained significant attention (Dobrowolski *et al.*, 2022, Mohamed *et al.*, 2023). Minerals such as calcium, phosphorus, zinc, and magnesium play vital roles in the body's physiological processes and may have significant impacts on the onset or progression of diseases (van Dronkelaar *et al.*, 2018). Phosphorus plays a critical role in various metabolic processes, including ATP production, DNA synthesis, and the regulation of cellular signaling. Consequently, disturbances in phosphate homeostasis are implicated in the development of MeTS (Mironov *et al.*, 2022, Wong, 2022). Hypophosphatemia may lead to reduced energy production and impaired glucose metabolism, thereby increasing the risk of obesity and hyperglycemia-both key components of MeTS. On the other hand, hyperphosphatemia has been associated with activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAAS), contributing to elevated blood pressure (Mironov *et al.*, 2022, Wong, 2022).

Similarly, calcium may influence MeTS through mechanisms parallel to those of phosphorus. These include modulation of intracellular signaling pathways to improve insulin sensitivity, inhibition of fatty acid synthesis, promotion of lipolysis, reduction of cholesterol levels, and suppression of RAAS activity leading to decreased blood pressure, as well as attenuation of inflammation and oxidative stress (Cheng *et al.*, 2019, Das and Choudhuri, 2021).

Like phosphorus and calcium, zinc is directly associated with insulin resistance and an increased risk of diabetes (Cunnane, 2018, Fukunaka and Fujitani, 2018). Additionally, normal serum zinc levels regulate and modulate inflammatory responses and immune cells, playing a role in reducing triglyceride and low density lipoprotein cholesterol (LDL-C) levels (Fukunaka and Fujitani, 2018). Therefore, the connection between this micronutrient and metabolic disorders and risk factors for MeTS is clear (Cunnane, 2018, Fukunaka and Fujitani, 2018). Magnesium is mainly involved in regulating the body's enzymatic reactions, impacting the health of the muscular, nervous, and cardiovascular systems (Fiorentini *et al.*, 2021). It has been shown that magnesium deficiency increases the risk of insulin resistance and diabetes, and supplementation with magnesium can reduce this risk (Kostov, 2019). Like the other minerals described, magnesium has beneficial antihypertensive and anti-inflammatory effects in the body (Fiorentini *et al.*, 2021, Kostov, 2019). Therefore, this study aims to provide evidence on the diagnostic and possibly prognostic value of these trace elements in identifying individuals at risk of metabolic syndrome, which may inform future nutritional or preventive strategies.

Materials and Methods

Design of study

This case-control study was conducted on patients with and without MeTS (Control group) and MeTS (Case group) who were visited in Yazd Diabetes Research Center and Khatam- Al-Anbia specialty clinic in Yazd by an endocrinologist in December 2020.

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Inclusion and exclusion

The inclusion criteria for the patient group were a diagnosis of MeTS (at least 3 out of 5 criteria of MeTS) and being within the age range of 20-60. The inclusion criteria for the control group were the absence of MeTS and an age range of 20-60 years. MeTS was diagnosed based on the following criteria (criteria were based on IDF/NCEP modified for Iranian population), and the presence of at least 3 out of 5 factors was considered (Azizi *et al.*, 2010): 1) Fasting plasma glucose ≥ 100 mg/dl or medication for controlling high blood glucose. 2) Blood pressure ≥ 130/85 mmHg or medication for controlling blood pressure. 3) Triglycerides ≥ 150 mg/dl or medication for lowering triglycerides. 4) For men, HDL-C ≤ 40 mg/dl, and for women, HDL-C ≤ 50 mg/dl or medication for increasing HDL-C. 5) Waist circumference (WC) in the Iranian population: ≥ 95 cm for men and women.

Exclusion criteria were intake of calcium, phosphorus, magnesium, and zinc supplements, use of diuretics, severe hyperglycemia, severe hypertension, pregnancy, lactation, vitamin D deficiency, thyroid disease, kidney disease, malignancy, acute infection, or individuals following specific diets.

Sample size

Based on the sample size calculation, a target of 60 participants per group was determined. The sample size was calculated using the two mean comparison formula. Considering type one error 0.05, effect size (d: 0.55) and power 90%, the calculated sample size included 149 participants, 79 diagnosed with MeTS and 70 healthy controls.

Measurements

All necessary data collection and clinical assessments were carried out by the researcher and documented using a standardized checklist. The collected data included demographic and anthropometric parameters such as age, sex, weight, height, BMI, WC, systolic (SBP) and diastolic blood pressure (DBP). In addition, a 12-hour fasting venous blood sample was obtained from each participant to measure fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), HDL-C, as well as serum concentrations of magnesium, calcium, phosphorus, and zinc. The levels of Mg, Ca, P and Zn were measured with Biosystem kits (Biosystems, Spain.), and all measurements were conducted with a BA400 Biosystem analyzer, following the manufacturer's protocols.

Ethical considerations

The study was conducted after obtaining the approval letter of the ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.REC.1393.198313), with the number 17/198313 on December 22, 2014. The patients were informed about the objective and nature of the study, and each participant provided written consent before the study.

Data analysis

Finally, data analysis was performed using SPSS-20 software. For quantitative data, the results were reported as mean±SD, and for qualitative data, as frequency (percentage). To compare the means of quantitative and qualitative variables between the two groups, after checking for normality of the data, one-way ANOVA, Chi-square test, and t-test were used. The significance level was considered as P-value<0.05.

Results

The study was conducted on 149 individuals, including 48 men (32.20%) and 101 women (67.80%), with a mean age of 42.20±2.13 years. Among them, 79 were patients with MeTS (53%) and 70 were healthy individuals (47%). There was no significant difference in gender between the groups (*P*=0.48).

Table 1 presents the mean serum levels of magnesium, calcium, phosphorus, and zinc in both groups and according to the number of MeTS factors. The results showed that the mean serum zinc level in healthy individuals was significantly higher than in patients with MeTS (*P*=0.02), but there were no statistically significant differences in the mean serum levels of phosphorus (*P*=0.11), calcium (*P*=0.09), and magnesium (*P*=0.51) between the two groups. Additionally, 12.8% of the

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individuals had at least one factor, and 17 individuals (11.40%) had all the 5 factors of MeTS. A comparison of these groups showed that with an increase in the number of MeTS factors, the serum zinc level significantly decreased (P=0.01). This difference was more pronounced in individuals with 4 or 5 MeTS factors. However, the increase in the number of MeTS factors did not have a significant impact on serum magnesium (P=0.98), calcium (P=0.06), and phosphorus (P=0.18) levels.

Table 1. Frequency distribution, mean±SD of serum zinc, magnesium, calcium, and phosphorus levels between the two groups and based on the number of MeTS factors.

Group	Mg (mg/dl)	Ca(mg/dl)	P(mg/dl)	Zn(mg/dl)
Control (n=70)	1.87 ± 0.38	10.0 ± 4.29	3.66 ± 0.52	88.83 ± 20.66
Case (n=79)	1.96 ± 0.98	9.20 ± 1.35	3.82 ± 0.56	84.36 ± 24.89
P-value[a]	0.51	0.09	0.11	0.02
0 Components of MeTS (n=19)	1.91 ± 0.42	9.57 ± 0.57	3.61 ± 0.52	91.7 ± 24.45
1 Components of MeTS (n=19)	1.84 ± 0.34	9.39 ± 0.85	3.58 ± 0.60	86.10 ± 15.29
2 Components of MeTS (n=32)	1.87 ± 0.40	10.63 ± 6.20	3.07 ± 0.48	88.70 ± 21.30
3 Components of MeTS (n=36)	1.95 ± 0.70	9.39 ± 1.42	3.78 ± 0.51	91.30 ± 27.17
4 Components of MeTS (n=26)	1.51 ± 0.24	9.04 ± 0.58	3.70 ± 0.57	76.60 ± 23.72
5 Components of MeTS (n=17)	3.51 ± 1.80	9.05 ± 1.90	4.07 ± 0.63	81.50 ± 17.60
P-value[b]	0.98	0.06	0.18	0.01

[a]: Student t-test; [b]: ANOVA test; Mg: Magnesium; Ca: Calcium; P: Phosphorus; Zn: Zinc; MeTS: Metabolic syndrome.

Table 2 shows that 89 individuals had normal blood pressure, while 60 individuals had high blood pressure (SBP≥130 or DBP≥85 mmHg). The serum zinc level in the hypertensive group was significantly lower than in the other group (P=0.02). There were no significant differences in the serum levels of magnesium (P=0.84), calcium (P=0.36), and phosphorus (P=0.10) between the two groups.

In this study, 102 individuals had TC levels below 200 mg/dl, and 43 had greater than or equal to 200 mg/dl. The difference in the mean serum levels of zinc (P=0.31), phosphorus (P=0.17), and calcium (P=0.08) between the two groups was not statistically significant. However, the serum magnesium level (P=0.04) in the high cholesterol group was significantly higher than in the normal cholesterol group. In this study, 62 individuals had normal HDL-C levels, while 87 had low HDL-C levels. The mean serum levels of zinc (P=0.002) and calcium (P<0.001) in the low HDL-C group were significantly lower than in the normal HDL-C group. The difference in the mean serum levels of magnesium (P=0.10) and phosphorus (P=0.27) between the two groups was not statistically significant (**Table 2**). In this study, 80 individuals had a waist circumference of less than 95 cm, and 69 individuals had a normal WC. The difference in the mean serum levels of zinc (P=0.08), calcium (P=0.29), magnesium (P=0.53) and phosphorus (P=0.14) between the two groups based on WC was not statistically significant (**Table 2**). In this study, 91 individuals had FBG levels higher than 100 mg/dl, and 58 individuals had normal levels. The difference in the mean serum levels of zinc (P=0.23), calcium (P=0.40), magnesium (P=0.77), and phosphorus (P=0.06) between the two groups based on FBG was not statistically significant (**Table 2**). In this study, 62 participants had TG levels higher than 150 mg/dl, and 87 had normal levels. The difference in the mean serum levels of zinc (P=0.70), calcium (P=0.26), magnesium (P=0.38), and phosphorus (P=0.29) between the two groups based on TG levels was not statistically significant (**Table 2**). **Table 2** showed a significant relationship between serum zinc (P=0.01) and calcium (P=0.01) levels and BMI, such that as BMI increased, the serum levels of zinc and calcium decreased. However, no significant relationship was observed between serum phosphorus (P=0.20) and magnesium (P=0.23) levels and BMI.

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Table 2. Comparison of serum levels of elements according to MeTS components.

Variable	Mg (mg/dl)	Ca (mg/dl)	P (mg/dl)	Zn (mg/dl)
Blood pressure(mmHg)				
Normotensive (N=89)	1.90 ± 0.41[b]	9.81 ± 3.92	3.69 ± 0.52	90.95 ± 21.76
Hypertensive (N=60)	1.94 ± 1.09	9.24 ± 1.13	3.82 ± 0.58	79.80 ± 23.43
P-value[a]	0.84	0.36	0.10	0.02
High density lipoprotein cholesterol (mg/dl)				
Normal (N=62)	1.90 ± 0.57	9.60 ± 0.70	3.67 ± 0.55	91.95 ± 24.66
Low (N=87)	1.88 ± 0.87	9.51 ± 4.04	3.80 ± 0.54	82.55 ± 21.08
P-value[a]	0.10	<0.001	0.27	0.002
Waist circumference (cm)				
Normal (N=69)	1.84 ± 0.41	10.0 ± 4.30	3.68 ± 0.54	88.53 ± 19.86
95 < (N=80)	1.99 ± 0.96	9.22 ± 1.33	3.80 ± 0.55	84.67 ± 25.44
P-value[a]	0.53	0.29	0.14	0.08
Fasting blood glucose (mg/dl)				
Normal (N=58)	1.90 ± 0.53	9.36 ± 1.08	3.64 ± 0.47	78.15 ± 24.52
100≤ (N=91)	1.93 ± 0.88	9.72 ± 3.89	3.81 ± 0.59	86.02 ± 22.16
P-value[a]	0.77	0.40	0.06	0.23
Triglycerides (mg/dl)				
Normal (N=87)	1.83 ± 0.38	9.76 ± 3.87	3.70 ± 0.54	84.88 ± 22.17
150≤ (N=62)	2.04 ± 1.08	9.32 ± 1.53	3.81 ± 0.56	88.67 ± 24.20
P-value	0.38	0.26	0.29	0.70
Body mss index classification (Kg/m^2)				
<18.5 (N=5)	2.00 ± 0.27	9.62 ± 0.77	3.44 ± 0.95	96.80 ± 27.15
18.5-25 (N=33)	2.02 ± 0.55	9.81 ± 0.80	3.62 ± 0.41	95.26 ± 28.54
25-30 (N=65)	1.81 ± 0.36	9.42 ± 0.66	3.75 ± 0.62	87.38 ± 18.79
30 < (N=46)	1.99 ± 1.21	9.17 ± 0.54	3.86 ± 0.46	77.72 ± 21.22
P-value[b]	0.23	0.01	0.20	0.01

*[a]: ANOVA test; [b]: Mean± SD; **Mg**: Magnesium; **Ca**: Calcium; **P**: Phosphorus; **Zn**: Zinc; **MeTS**: Metabolic syndrome.*

Discussion

Minerals and trace elements regulate various physiological functions in the body, and their normal serum levels are essential for maintaining overall health. Abnormal levels in any of these elements can affect multiple physiological reactions and increase the risk of various diseases. It has been established that disturbance of these vital elements plays a role in diabetes, insulin resistance, obesity, and cardiovascular diseases, all of which are directly or indirectly related to MeTS. Examining the changes in the serum and urine levels of these elements in patients can provide valuable information on their role in different diseases (Dubey *et al.*, 2020). This study aims to determine the relationship between serum levels of these minerals and MeTS. The results of the present study indicated that serum zinc levels were significantly lower in the MeTS group, while serum calcium, phosphorus, and magnesium levels did not show statistically significant differences between the two groups. Furthermore, serum zinc levels significantly decreased as the number of MeTS factors increased, especially with 4 or 5 factors. In some studies, no significant difference was reported between zinc and MeTS (Fang *et al.*, 2019, Obeid *et al.*, 2008). However, numerous studies have shown a close relationship between serum zinc levels and MeTS and its components (Lu *et al.*, 2021, Yeung *et al.*, 2009). They reported that serum zinc levels were significantly higher in the control group compared to the MeTS group; similar to this study, serum zinc levels were inversely and directly related to the number of MeTS components (Lu *et al.*, 2021, Yeung *et al.*, 2009). As the number of components of this syndrome increased, serum zinc levels decreased more in patients (Lu *et al.*, 2021, Yeung *et al.*,

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2009). Based on these results, zinc is one of the key elements involved in MeTS, and an adequate intake of zinc-containing foods or zinc supplements is crucial for the prevention and treatment of MeTS. Several studies have reported the positive effects of zinc supplementation in the treatment of this syndrome (Althanoon and Merkhan, 2021, Ruz *et al.*, 2019). Althanoon *et al*. in 2021 demonstrated that consuming a 50 mg zinc tablet per day for three months improved lipid profile, blood glucose, BMI, and blood pressure in patients with MeTS (Althanoon and Merkhan, 2021). In the case of magnesium, some studies have reported a direct relationship between serum magnesium levels and MeTS (Yuan *et al.*, 2016), while others have reported an inverse relationship (La *et al.*, 2016). Although some studies have shown that higher magnesium consumption and supplementation are associated with a reduced risk of developing this syndrome and may help in its treatment (Guerrero-Romero *et al.*, 2016, Sarrafzadegan *et al.*, 2016), further studies, particularly in the form of cohort studies, are recommended (Sarrafzadegan *et al.*, 2016). The relationship between calcium, phosphorus, and MeTS is limited and debated, with studies providing varying results (Jhuang *et al.*, 2019, Osadnik *et al.*, 2020, Yuan *et al.*, 2016). Jhuang *et al*. demonstrated that the relationship between phosphorus and MeTS was age-dependent, reporting that serum phosphorus levels in individuals over 60 were associated with MeTS, but no significant relationship was found in individuals under 60 years of age (Jhuang *et al.*, 2019). A 4.3-year retrospective longitudinal study on 12,706 people showed that there was no positive relationship between serum calcium levels and the risk of MeTS. However, the risk of developing this syndrome was lower in individuals with central obesity who had higher serum calcium levels (Baek *et al.*, 2017). Based on these studies, it seems that zinc level is related to MeTS, but more studies with a large sample size are needed to assess the exact relationship of zinc and other elements with MeTS. Also, randomized clinical trial with the purpose of supplemental or dietary zinc intake for evaluating the incidence of MetS and its components may be helpful. An effective approach to the reduction of metabolic syndrome may be the focus of dietary therapy and exercise. The strength of this study is that it is the first research that evaluated and compared the level of this trace element in two groups in Yazd with high prevalence of obesity and metabolic syndrome. There were some limitations to this study. First, due to the cross-sectional design, determining the cause and effect of the observed associations was impossible. The second limitation was small sample size and a lack of dietary data to assess the amount of mineral intake.

Conclusion

Lower serum zinc levels are significantly associated with the presence and severity of metabolic syndrome, particularly in relation to elevated blood pressure and reduced HDL-C levels. While calcium, phosphorus, and magnesium levels showed no consistent associations, zinc appears to play a more prominent role in metabolic regulation. Although these results suggest the potential value of zinc supplementation as a therapeutic adjunct in MeTS management, further longitudinal and interventional studies are required to confirm its clinical efficacy and safety.

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Authors' contributions

Mirhosseini SS wrote the manuscript, revised and edited the manuscript, prepared the submission, and handled all correspondence with the journal including responding to reviewers' comments and emails. Ghadiri-Anari A supervised of the project. provided scientific guidance, oversight, and critical review of the study and manuscript. Zohal M and Moosavi A wrote the manuscript, contributed to data collection and analyzed them. All authors approved the final version of the manuscript.

Conflict of interests

The authors declared no competing interests.

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