Association between Serum Zinc and hs-CRP Concentrations in Different Metabolic Syndrome Phenotypes

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Abstract

**Background:** Metabolic syndrome (MetS) is associated with an increased risk of cardiovascular disease and all-cause mortality. Zinc (Zn) is an essential trace element for the synthesis, storage, and release of insulin. The relationship between serum Zn and MetS is controversial. In this study we have evaluated whether serum Zn levels are associated with hsCRP level according to MetS phenotypes.

**Method:** A total of 9493 individuals (3768 men and 5635 women) were recruited as part of the Mashhad Stroke and Heart Association Disorder (MASHAD) study. We measured the concentration of serum Zn by flame atomic absorption (Varian AA240FS) and we divided the subjects into quartile of serum Zn. We used SPSS version 18 statistical analyses for all. GraphPad Prism 6 for figures was used.

**Results:** In this study there were significant differences between quartiles of Zn according to sex. The results showed that the level of serum hs-CRP were higher in subjects with metabolic syndrome who also had a serum Zn >95 µg/dl. In subjects with serum Zn <70 µg/dl, an increase in serum hs-CRP was associated with an increased risk of MetS by 2.2%, 1.022(CI: 1.01-1.034). Moreover, among subjects in the first, second and third quartiles of serum Zn, some phenotypes of metabolic syndrome (WHB, WHT, WTG, WTB, WHBG, WHTG, and WTBG) were associated with a risk of increasing hs-CRP level (p< 0.05).

**Conclusion:** Serum hs-CRP concentrations were related to MetS phenotypes. This relationship was modified by serum Zn level. According to the number of MetS components serum Zn levels decreased as the number of MetS component increased, and low serum zinc levels (Q1) showed an overall greater prevalence of MetS.

1. Introduction

Metabolic syndrome (MetS), a cluster of metabolic risk factors including glucose intolerance, atherogenic dyslipidemia, elevated blood pressure and abdominal obesity, is associated with an increased risk of cardiovascular disease (CVD) and all-cause mortality (1–3). The prevalence of MetS has been increasing rapidly worldwide (4) and this has become a major medical concern.

Zinc (Zn) is an essential trace element, involved in the synthesis, storage, and release of insulin(5, 6). This mineral is also a fundamental component required for the synthesis of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase(7). Therefore, Zn deficiency may induce abnormal insulin metabolism and oxidative stress, which are important factors for the pathophysiology of diabetes and metabolic syndrome (MetS) (8–10).

The relationship between serum Zn and MetS is controversial. Higher levels of dietary Zn have been reported to be protective against the development of MetS in specific populations (11, 12). An inverse relationship between serum Zn concentration and MetS was also reported in women (13). In contrast, a longitudinal study reported that higher serum Zn concentration are a predictive factor for MetS (14).

The potential role of oxidative stress and chronic inflammation in MetS has also been reported, and increased oxidative stress or the presence of chronic inflammation may affect the development of MetS (15–17). Despite the critical roles of insulin resistance and/or oxidative stress and chronic inflammation in MetS pathogenesis (16, 17) and the functions of Zn related to insulin resistance (7, 8), oxidative stress (9), or chronic inflammation (10, 13), studies on the link between MetS and body zinc status are scarce and the results are controversial.
Furthermore, to our knowledge the association between MetS phenotypes and serum Zn levels is conflicting. Therefore, we evaluated whether or not serum Zinc levels are associated with hsCRP level according to MetS phenotypes.

2. Method

2.1 Study population

In this study 9403 individuals (3768 men and 5635 women) were investigated as part of the Mashhad Stroke and Heart Association Disorder (MASHHAD) study. They were initially selected using a random clustering method during the period of 2007–2008, as previously described (18). The full agreement of all participants was taken using the protocols approved by the Mashhad University of Medical Sciences (MUMS) Ethics Committee. The criteria for entering and leaving the study of MASHAD and the general characteristics of sample population such as marital and occupational status, educational level, drug use, biochemical measurements and anthropometrics was reported previously (18). Several biochemical characteristics of participants were measured at baseline. Then serum samples were aliquoted into 0.5 mL tube and stored at -80°C. The subjects were then divided into groups with or without MetS, defined by the IDF criteria (19). In this study we divided participants into groups, metabolic syndrome (3398 (28.4%)) and healthy (6005 (51.4%)) subjects, then we measured serum Zn in all subjects. We were also able to define eleven metabolic syndrome phenotypes based on these criteria: WHG (waist circumference, HDL, glucose), WHB (waist circumference, HDL, blood pressure), WHT (waist circumference, HDL, TG), WBG (waist circumference, blood pressure, glucose), WTG (waist circumference, TG, glucose), WTB (waist circumference, TG, blood pressure), WHBG (waist circumference, HDL, blood pressure, glucose), WHTG (waist circumference, HDL, TG, glucose), WHTB (waist circumference, HDL, TG, blood pressure), WTBG (waist circumference, TG, blood pressure, glucose) and WHTBG (waist circumference, HDL, TG, blood pressure, glucose) according to IDF criteria.

2.2 Demographic, anthropometric and metabolic data

For all the participants, systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), serum high-density lipoprotein cholesterol (mg/dl), triglycerides (mg/dl), blood glucose (mg/dl), waist circumference (cm), high-sensitivity C-reactive protein (mg/l) and zinc intake (mg/day) were measured. Waist circumference was measured with a tape measure to the nearest millimeter. Systolic and diastolic blood pressure (SBP and DBP) was measured twice using the same standard sphygmomanometer. Biochemical parameters such as high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-sensitivity C-reactive protein (hsCRP) and serum glucose were determined by the methods describe previously (18).

2.3 Measurement of serum zinc

Sera were diluted with nitric acid 1.5% at the ratio of 1:10. The concentration of serum Zn was measured with Flame atomic absorption (Varian AA240FS). Zn standard curves were made using Zn standard (Merck and Pharmacy Company). The accuracy of the methods for Zn was 93 ± 4.8%, which was evaluated through measuring the confirmed reference material (Merck KGaA 64271 Darmstadt, Germany) comprising known values (1000 ± 2 mg / L) Zn. Also for Zn the intra-assay and inter-assay coefficient of variation (CV) were 1.5 ± 0.2% and 2.6 ± 0.4%.

Participants were divided into different groups based on serum zinc. Serum zinc was defined as quartiles and based on this we investigated various factors in different zinc groups.

2.4 Statistical analysis
All statistical analyses were undertaken using SPSS version 18 (SPSS Inc. Chicago, IL, USA). The normality of the data was assessed using the Kolmogorov-Smirnov test. Descriptive statistics including mean, frequency, and standard deviation (SD) were defined for all variables and expressed as mean ± standard deviation (SD) for variables with normally distribution or median ± IQR for not normally distributed variables. One-Way ANOVA or Kruskal-wallis H was determined. Bonferonni corrections were made for multiple comparisons. Chi-square or Fisher exact tests were used to evaluate the categorical parameters. Logistic regression analysis with sex included as model covariates was used to evaluate the relation between increasing hs-CRP level in MetS according to zinc quartiles. All the analyses were two-sided and P values less than or equal to 0.05 were considered significant. GraphPad Prism 6 for figures was used.

3. Result

3.1 General characteristics of the subjects

Of the total group (9493), 3768 were male and 5635 were female, and there were significant differences between the quartiles of zinc according to sex (p < 0.05) (Table 1).
|                                      | Q1(< 70 µg/dl) | Q2(70–78 µg/dl) | Q3(78–95 µg/dl) | Q4(> 95 µg/dl) | p-value |
|--------------------------------------|----------------|----------------|----------------|----------------|---------|
| Prevalence % of MetS criteria        | 9493(100)      | 2205(22.4%)    | 2641(26.9%)    | 2270(23.1%)    | 2377(24.2%) |
| (population frequency)               |                |                |                |                |         |
| Age, y                               | 48.24 ± 7.94   | 48.35 ± 8.1    | 47.82 ± 7.81   | 47.23 ± 6.91   | 0.71    |
| Sex, %                               |                |                |                |                |         |
| Male(3768), %                        | 860(22.8%)     | 1033(27.4%)    | 872(23.3%)     | 1003(26.6)     | 0.028   |
| Female(5635), %                      | 1333(23.7%)    | 1581(28.1%)    | 1374(24.4%)    | 1347(23.9)     |         |
| SBP, mm Hg                           | 121.06 ± 17.72 | 121.68 ± 19.24| 122.2 ± 18.95  | 121.35 ± 20.65 | 0.59    |
| DBP, mm Hg                           | 78.06 ± 11.6   | 79.04 ± 11.82  | 79.21 ± 11.74  | 78.66 ± 12.25  | 0.038   |
| HTN (SBP 130 mm Hg and/or DBP 85 mm Hg and/or medication use), % | 3792 (40.8%) | 893(23.5%) | 1002(26.4%) | 939(24.1%) | 958(25.3%) | 0.1 |
| HDL-C, mg/dl                         | 42.2 ± 10.86   | 42.68 ± 9.82   | 43.72 ± 10.07<sup>ab</sup> | 42.66 ± 9.82<sup>c</sup> | < 0.001 |
| HDL-C 40 mg/dl for men or 50 mg/dl for women, % | 6184(66.2%) | 1506(24.4%) | 1751(28.3%) | 1408(22.8%)<sup>ab</sup> | 1519(24.6%)<sup>ab</sup> | < 0.001 |
| Triglycerides, mg/dl                 | 122(86-177.5)  | 121(84-177.25) | 118(84–170)    | 121(86–169)    | 0.49    |
| Triglycerides 150 mg/dl, %           | 3183(34.1%)    | 777(24.4%)     | 906(28.5%)     | 726(22.8%)     | 774(24.3%) | 0.091 |
| Glucose, mg/dl                       | 93.47 ± 41.41  | 92.06 ± 40.1   | 92.06 ± 33.75  | 93.01 ± 38.93  | 0.94    |
| Glucose 100 mg/dl and/or antidiabetic medication use, % | 1738(18.6%) | 397(22.8%) | 472(27.2%) | 418(24.1%) | 451(25.9%) | 0.74 |
| waist circumference (cm)             | 95.88 ± 12.01  | 95.06 ± 11.95  | 95.78 ± 12.23  | 94.78 ± 12.06<sup>c</sup> | 0.039 |

<sup>a</sup>p < 0.05 compared to Q1.<nsup>b</sup>p < 0.05 compared to Q2.<sup>c</sup>p < 0.05 compared to Q3.
Demographic and metabolic characteristics of the study population according to quartiles of Zinc is presented in Table 1. The mean age of the quartile for serum Zn were 48.24 ± 7.94 (Q1 (< 70 µg/dl)), 48.35 ± 8.1 (Q2 (70–78 µg/dl)), 47.82 ± 7.81 (Q3 (78–95 µg/dl)) and 47.23 ± 6.91 (Q4 (> 95 µg/dl)), respectively. The results indicated that in subjects who had serum zinc between 78 and 95, DBP and HDL-C was higher and there was a significant difference between these groups (p < 0.05). However, in subjects with serum zinc > 95 µg/dl waist circumference was lower and hs-CRP was significantly higher than other groups (p < 0.05) (Table 1).

### 3.2 Serum hs-CRP level according to Zinc Quartiles in Metabolic Syndrome

Figure 1, shows serum hs-CRP level for the individuals with and without metabolic syndrome according to serum Zn quartiles. The second and third quartiles were merged because they had similar values and were within normal range. The serum hs-CRP was higher in subjects with MetS in forth quartile (zinc > 95 µg/dl) (p < 0.05) (Fig. 1).

Serum hs-CRP levels in WHB, WBG, WTB, WHTB, WTBG and WHTBG phenotypes of metabolic syndrome was significantly higher versus other phenotypes (p < 0.05) (Table 2) in the first quartile; in second and third quartile WTB, WHTG, WHTB and WHTBG had the higher level of hs-CRP (p < 0.05); and in forth quartile WHT had the higher level of hs-CRP (p < 0.05) (Table 2).
Table 2
hsCRP level in phenotypes of Metabolic Syndrome according to quartiles of Zinc

|       | Q1             | Q2,3            | Q4            |
|-------|----------------|-----------------|---------------|
| WHG*  | 1.58(1.01–3.4) | 1.56(0.93–3.19) | 1.62(1.08–4.26) |
| WHB   | 2.18(1.23–4.4) | 2.03(1.12–3.84) | 2.29(1.21–5.18) |
| WHT   | 1.88(1.16–4.16)| 2.01(1.13–3.84) | 1.83(1.3–3.96) |
| WBG   | 1.79(1-7.3)    | 1.92(1.21–5.78) | 2.10(1-10.2)  |
| WTG   | 2.29(1.32–8.26)| 1.93(1.02–4.31) | 1.63(1.22–3.59) |
| WTB   | 4.19(1.3–6.16) | 2.69(1.23–5.22)| 2.95(2.03–4.39) |
| WHBG  | 1.56(1.17–4.22)| 1.99(1.16–3.24) | 2.60(1.62–6.3) |
| WHTG  | 2.09(1.19–5.5) | 2.40(1.28–4.79)| 2.54(1.43–6.69) |
| WHTB  | 2.27(1.31–3.8)| 2.26(1.3–3.85)| 2.62(1.4–6.1) |
| WTBG  | 6.20(2.792)    | 4.38(1.45–7.65)| 1.65(0.81–2.14) |
| WHTBG | 2.10(1.38–4.26)| 3.05(1.5–6.3)| 2.43(1.4–4.57) |
| p-value| < 0.001        | < 0.001         | 0.016         |

P < 0.05, a: WHG vs another’s; b: WHB vs another’s; c: WHT vs another’s; d: WTB vs another’s; W: waist circumference; H: HDL; T: Triglyceride; B: Blood pressure; G: Glucose

Data presented by interquartile range, One-Way ANOVA has been done; Q1 < 70 µg/dl, Q2 and Q3 pooled (70–95 µg/dl) and Q4 > 95 µg/dl

*: hs-CRP compared in every metabolic syndrome phenotypes in different zinc quartiles

**3.3 Risk of increasing hs-CRP level according to Zinc Quartiles in Metabolic Syndrome**

In subjects with a serum zinc < 70 µg/dl, an increase in serum hs-CRP was associated with an elevated risk of MetS (an increase of one unit (1 mg/l) increased the chance of MetS by 2.2% time (OR: 1.022 (CI: 1.01–1.034)) (p < 0.05), subjects with zinc between 70–95 µg/dl, an increase of serum hs-CRP by one unit (1mg/l) can increase the chance of getting metabolic syndrome by 1.1% time (OR: 1.011 (CI: 1.006–1.019) (p < 0.05) in serum hs-CRP level in metabolic syndrome (Fig. 2).

Moreover, among subjects in the first quartile of serum Zn, some phenotypes of metabolic syndrome (WHB, WHT, WTG, WTB, WHBG, WHTG, and WTBG) were associated with a risk of increasing hs-CRP level (p < 0.05) (Table 3). In the second and third quartiles of zinc WHB, WHT, WBG, WTB, WHTG, WTBG, and WHTBG and in the fourth quartile WHG had risk of rising hs-CRP level in metabolic syndrome (p < 0.05) (Table 3).
Table 3
Risk of increasing hs-CRP in Metabolic Syndrome Phenotypes according to Zinc Quartiles. Multivariate adjusted odds ratios (95% CI) were calculated using logistic regression with sex included as model covariates.

|     | Q1                          | Q2,3                        | Q4                          |
|-----|-----------------------------|-----------------------------|-----------------------------|
| WHG | 1.019(0.991–1.049)          | 1.002(0.975–1.029)          | 1.022(1.004–1.04)\*         |
| WHB | 1.036(1.01–1.062)\*         | 1.023(1.016–1.049)\*        | 1.012(0.988–1.038)          |
| WHT | 1.026(1.005–1.047)\*        | 1.024(1.01–1.038)\*         | 1(0.983–1.018)              |
| WBG | 1.03(0.098–1.083)           | 1.042(1.014–1.071)\*        | 1.025(0.982–1.068)          |
| WTG | 1.046(1.005–1.088)\*        | 1.023(0.982–1.066)          | 0.945(0.83–1.076)           |
| WTB | 1.055(1.028–1.082)\*        | 1.043(1.022–1.064)\*        | 1.01(0.968–1.053)           |
| WHBG| 1.046(1.01–1.085)\*         | 1.022(1.01–1.085)           | 1.008(0.948–1.072)          |
| WHTG| 1.033(1.006–1.06)\*         | 1.036(1.019–1.054)\*        | 1.007(0.978–1.036)          |
| WHTB| 1.023(0.994–1.053)          | 1.029(1.012–1.047)\*        | 1.007(0.983–1.031)          |
| WTBG| 1.056(1.009–1.106)\*        | 1.049(1.02–1.1079)\*        | 0.709(0.313–1.031)          |
| WHTBG| 1.011(0.949–1.073)          | 1.044(1.0129–1.066)\*       | 1.02(0.89–1.062)            |

*P < 0.05 ; W: waist circumference; H: HDL; T: Triglyceride; B: Blood pressure; G: Glucose; reference group was without any component of metabolic syndrome

4. Discussion
Serum hs-CRP concentrations were related to the phenotypes of MetS defined by IDF criteria. This relationship was modified by serum zinc concentrations. According to the number of MetS component serum Zn levels decreased as the number of MetS component increased, and low serum zinc levels (Q1) showed greater prevalence of MetS (40.4%). Serum Zn concentrations are viewed as an appropriate indicator for evaluating zinc status, although the sensitivity and specificity of the serum zinc level may be affected by confounding factors such as acute stress and infection (20). In agreement with our study, Jin-A Seo showed that with increasing metabolic syndrome components, serum Zn levels fell (21). Also, Freitas et al showed that serum zinc and zinc uptake in subjects with MetS are lower than healthy subjects and they had adequate zinc and moreover, an increase urinary zinc excretion (22).

Mean serum zinc in a population may reflect the status of dietary zinc intakes, and could be used as an indicator of zinc deficiency of the population (23).

The relationship between serum Zn and other metabolic parameters is controversial. Several studies have evaluated the association of MetS with serum Zn concentrations. In a few studies, men and women with MetS had a higher and lower level of serum zinc than those without, respectively (1, 3, 23). In contrast, other studies reported no relationship between serum zinc concentration and MetS (1, 2). Other factors not included in the clinical
definition of MetS, such as chronic inflammation or oxidative stress, may lead to the development of MetS (15, 16). Inflammatory cytokines released tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), stimulate C-reactive protein production in the liver, during the MetS (17).

Zn a cofactor for antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, decrease reactive oxygen species (ROS) generation and induces metallothionein, which decreases the burden of ROS (1), suggesting that a decrease in body zinc status may contribute to the development of MetS. In addition, chronic inflammation or oxidative stress may contribute to the decreased serum zinc levels.

The relationship between serum Zn and hs-CRP with MetS was investigated in previous studies, but the strength of this current study was the sample size, and the assessment of serum Zn and hs-CRP in different MetS phenotypes. Although, one of the limitation of this study is the cross-sectional, design so we need more cohort study to confirm decreasing zinc level can elevated hs-CRP concentration according to metabolic syndrome components.

Declarations

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Declaration of interest:

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.REC.1397.479), and informed consent was obtained from all participants.

Consent for publication:

All authors agree.

Availability of data and material:

None

Competing interests:

None
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Authors' contributions
Susan Darroudi: Study design, data collection and data analysis
Batool Tayefi, Fatemeh Sadabadi, Hamideh Moalemzadeh Haghighi: write paper
Habibollah Esmaily: Study design and data analysis
Amir Hooshang Mohammadpour: scientific advice
Gordon A. Ferns: study design and English editing
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Figures
serum hs-CRP level according to serum zinc quartiles in individuals with Metabolic Syndrome; (Negative: No Metabolic Syndrome, Positive: Metabolic Syndrome); Mann-Whitney test has been done; * p< 0.01

Figure 1

Zinc Quartiles in Metabolic Syndrome; * p< 0.01
Figure 2

Risk of an high serum hs-CRP in Metabolic Syndrome according to serum Zn quartiles. Adjusted odds ratios (95% CI) were calculated using logistic regression with sex included as model covariates. *p<0.05