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*Lupus* published online 23 April 2014
DOI: 10.1177/0961203314530792

Lupus (2014) 0, 1–9
http://lup.sagepub.com

PAPER

# Association between serum trace element concentrations and the disease activity of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease with a complex, incompletely understood, etiology. Several genetic and environmental factors are suspected to be involved in its aetiology. Oxidative stress may be implicated in the pathogenesis of SLE and may be affected by trace element status. Zinc (Zn), copper (Cu) and selenium (Se) are essential components of several anti-oxidative enzymes and are also involved in several immune functions. The current study aimed to assess the relationship between serum concentrations of these trace elements and the clinical disease activity of SLE assessed using the SLE disease activity index (SLEDAI). Serum concentrations of albumin (Alb) ($p = 0.001$), Se ($p = 0.001$), Zn ($p = 0.001$) and the Zn to Cu ratio (Zn/Cu R) ($p = 0.001$) were lower in patients with SLE than the age- and sex-matched healthy controls. However, only Alb ($p = 0.001$) and Cu ($p = 0.03$) were negatively correlated with disease activity, which was supported by regression analysis. In summary, lower serum values of Alb, Zn, Se and Zn/Cu R were found in SLE patients compared with healthy controls; however, in addition to serum Alb concentrations, serum Cu concentrations were also negatively correlated with lupus disease activity. *Lupus* (2014) **0,** 1–9.

**Key words:** SLE disease activity index (SLEDAI); systemic lupus erythematosus (SLE); trace element; zinc; copper; selenium; ceruloplasmin; zinc to copper ratio

## Introduction

Systemic lupus erythematosus (SLE) is an important multi-system autoimmune disease, which can affect the kidneys, heart and central nervous system.[1–2] Oxidative stress is one factor that may exacerbate disease activity. SLE usually presents with a fluctuating clinical course, with occasional flare-ups and remissions. There appears to be exogenous and endogenous determinants that interact with genetic susceptibility to orchestrate disease development and progression; one important component in this scenario is oxidative stress.[3]

Trace elements such as zinc (Zn), copper (Cu) and selenium (Se) play a crucial role in maintaining the oxidative defense of cells.[4] Zn takes part in the modulation of several cellular interactions including DNA and protein synthesis, cell proliferation, electron transport and defense against free-radical damage.[5] Prasad[6] has suggested that Zn has both anti-inflammatory and antioxidant effects. Cu is a central component of the antioxidant enzyme superoxide dismutase. It is also an essential component of ceruloplasmin (Cp) which protects cells from free-radical injury. Ferns et al.[7] have proposed both pro- and anti-oxidative in-vivo effects of Cu. Se is an essential constituent of anti-oxidative enzymes including selenoprotein P, glutathione peroxidases and thioredoxin reductases which are responsible for Se transport (selenoprotein P), antioxidant/redox properties (glutathione peroxidases (GPxs), thioredoxin reductases and selenoprotein P) and anti-inflammatory properties (selenoprotein S and GPx4).[8]

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Received 21 August 2013; revised: 18 November 2013
accepted 11 March 2014


10.1177/0961203314530792

Rheumatoid arthritis has previously been investigated with respect to the role of trace elements in disease activity,[9–11] and there are limited data available concerning lupus.[12] Because of the potential importance of trace elements in the pathogenesis of SLE, and especially the vital role of Zn, Cu and Se in oxidative defense, the current study was designed to evaluate the relationship between serum values of these trace elements and lupus disease activity in addition to disease manifestations.

## Materials and methods

### *Study population*

A case-control, cross-sectional study was undertaken with 223 participants including 123 patients, who were classified as having SLE using the American College of Rheumatology criteria,[13] and 100 healthy controls from the friends of patients and medical staff; they were selected by interview and physical examination and recruited from the Rheumatic Diseases Research Center (RDRC), Mashhad, Iran.

The exclusion criteria for both groups were as follows: any other autoimmune disease, intake of nutritional supplements including trace elements in the six months prior to the blood collection, any condition that may be associated with malnutrition including chronic diarrhoea, Body mass index (BMI) I $\leq$ 18, cholesterol $<$100 mg/dl and albumin (Alb) $<$2 mg/dl. Moreover, patients with a glomerular filtration rate (GFR) $<$80%, those with concomitant infection or other diagnosed chronic or overlapping collagen diseases or who were pregnant or in the breastfeeding period were also excluded. Among the patients, 53.7% were treated with prednisolone at the time of the study. The average dosages of prednisolone according to disease activity were 5(5) mg/d and 10(22.5) mg/d for patients with inactive and active disease respectively. Approximately 20% of patients were treated with cytotoxic drugs including azathioprine, mycophenolate mofetil or cyclophosphamide prior to blood sampling. Blood sampling was performed before starting treatment in new cases, or before increasing doses of prednisolone or cytotoxic drugs or dispensing new drugs to treat relapses or new organ involvements in patients with established SLE.

Each volunteer gave informed written consent for participation in the study, and the study protocol was approved by the Mashhad University of Medical Science Ethics Committee. Cp, Alb and trace elements including Zn, Cu and Se were determined in both the control and case groups. Other laboratory parameters including anti-double stranded DNA (anti-dsDNA), complement serum values including C3, C4, creatinine, full blood count, urinary sedimentation (red blood cell count (RBC) or white blood cell count (WBC) cast) and 24 h urine protein excretion were measured in all patients.

### *Disease activity index*

Disease activity was assessed in patients with SLE according to the SLEDAI2K (10). The SLE disease activity index (SLEDAI) is a global score developed for the assessment of SLE disease activity. Patients were divided into two groups using the SLEDAI; those with a SLEDAI $\leq$4 and those with a SLEDAI $>$4.

### *Trace element measurement*

Serum Cu and Zn were measured by flame atomic absorption spectrometry (Varian, USA). Serum Se was determined by electrothermal atomic absorption spectrometry with Zeeman background correction using a palladium chloride chemical modifier.[14] Wave lengths used for analyzing Cu, Zn and Se were 324, 213 and 196 nm respectively. Typical between batch precision (CVs) for these assays were 3.6 and 2.7% respectively.

Cp was measured by radial immuno-diffusion,[14] as previously described. The between assay CV was typically 3.8%.

### *Statistical analysis*

After using a Klomogrov–Smirnov test for normality, parametric or nonparametric tests were utilized as appropriate to analyze the data. Normally distributed parameters were reported as mean ($\pm$ standard deviation (SD)) and nonparametric variables were reported as mean (with inter-quartile range). Student *t*-test, one-way analysis of variance (ANOVA) and Pearson correlation tests were applied to analyze normal distributed data and Mann-Whitney, Kruskal-Wallis and Spearman correlation tests were also used to analyze nonparametric data. For some parameters logarithmic transformation was used to normalize values (Cp and SLEDAI). Cut-off values for trace elements between patients and healthy volunteers were selected using MedCalc software 11.5.1. Positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), sensitivity, specificity and Youden's index for the estimated cut-off points were calculated

using MedCalc software 11.5.1. Statistical analysis was performed using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA).

## Results

### *Demographics*

Table 1 presents the demographic data of the participants. The frequency of patients with active disease (SLEDAI >4) and patients with inactive disease (SLEDAI ≤4) was 63.1% and 36.9% respectively. The mean of SLEDAI was an 8(13) score. Important clinical features of disease according to SLEDAI in the SLE group were as follows: malar rash (48%), alopecia (12%), arthritis (38.8%), myositis (3.4%), cytoid body (1.8%), lupus related fever (%4), hemolytic autoimmune anemia (9.9%), leucopenia (52%), thrombocytopenia (22.1%), proteinuria (21%), increased anti-dsDNA (43%), decreased C3 complement (36.5%), decreased C4 complement (41.3%), central nervous system (CNS) involvement (13%) and cardiac involvement (1.7%).

### *Comparison of biochemical markers and trace elements between patients with lupus and healthy controls*

Serum Zn, Alb, Se and Zn to Cu ratio (Zn/Cu R) were significantly lower in patients compared with healthy controls ($p < 0.05$, Table 1).

### *Correlation of biochemical markers and trace elements with disease activity*

Comparison of those biochemical markers between patients with active and inactive disease based on SLEDAI scores demonstrated that only Alb was significantly lower in the active disease group; whereas, Zn, Cu, Se and Cp values were at similar concentrations in these two patient groups (Table 2). However, when Pearson's correlation analysis was conducted on the data, among the measured parameters, serum Alb and Cu were negatively correlated with disease activity (Table 3).

### *Comparison of biochemical markers and trace elements between patients with or without proteinuria and those correlations with anti-dsDNA, C3 and C4*

Serum Alb, Cu and Zn were significantly lower in patients with proteinuria, while serum Se, Cp and Zn/Cu ratio (R) did not differ significantly between patients with and without proteinuria (Table 4). A positive correlation between 24 h urinary protein excretion and serum Se concentrations and a negative correlation between proteinuria and serum Alb, Zn and Cu levels were found (Table 5). There were no significant correlations between

**Table 1** Comparison of demographic and biochemical measures between patients with lupus and healthy subjects

| | *Case group (n = 100)* | *Control group (n = 123)* | p *value* |
|---|---|---|---|
| Age (year) | 27 (11.25) | 25 (18) | 0.15, $z = -0.14$ |
| Gender (% female) | 90% | 91.1% | 0.7, *chi* = 0.08 |
| Disease duration (year) | 5 (7) | - | - |
| Alb (g/dL) | 4.35 (1.4) | 4.8 (0.6) | 0.001, $z = -5.4$ |
| Cu (μg/L) | 935.68 ± 218.80 | 911.08 ± 240.45 | 0.4, $t = -0.7$ |
| Se (μg/L) | 98.90 ± 18.43 | 124.92 ± 21.09 | 0001, $t = 9.3$ |
| Zn (μg/L) | 700.61 ± 135.91 | 860.45 ± 123.74 | 0.001, $t = 8.7$ |
| Cp (mg/L) | 401.17 ± 184.29 | 369.8 ± 116.91 | 0.12, $t = 8.7$ |
| Zn/Cu | 0.74 (0.22) | 0.97 (0.42) | 0.001, $z = -5.8$ |

**Table 2** Comparison of biochemical measurers and trace elements between patients with lupus and healthy subjects

| | *Case group* | *Control group* | p *value* |
|---|---|---|---|
| Alb (g/dL) | 4.35 (1.4) | 4.8 (0.6) | 0.001, $z = -5.4$ |
| Cu (μg/L) | 935.68 ± 218.80 | 911.08 ± 240.45 | 0.4, $t = -0.7$ |
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| Zn/Cu | 0.74 (0.22) | 0.97 (0.42) | 0.001, $z = -5.8$ |

**Table 3** Correlations between biochemical parameters and disease activity

| | *SLEDAI* | |
|---|---|---|
| | *Correlation coefficient* | p *value* |
| Alb (g/dL) | $r_p = -0.4$ | 0.001 |
| Cu (μg/L) | $r_p = -0.2$ | 0.03 |
| Se (μg/L) | $r_p = 0.1$ | 0.2 |
| Zn (μg/L) | $r_p = -0.1$ | 0.1 |
| Cp (mg/L) | $r_p = -0.1$ | 0.2 |
| Zn/Cu R | $r_s = 0.04$ | 0.6 |

**Table 4** Comparison of biochemical parameters and trace elements between patients with and without proteinuria

| *Biochemicals* | *Patients with Proteinuria* | *Patient without Proteinuria* | p *value* |
|---|---|---|---|
| Serum Alb (g/dL) | 3.15 (2.03) | 4.5 (1) | 0.002, $z = -3.1$ |
| Cu (μg/L) | 855.7 ± 150.4 | 969.9 ± 237.6 | 0.01, $t = -2.6$ |
| Se (μg/L) | 98.2 ± 16.8 | 99.63 ± 18.8 | 0.74, $t = -0.3$ |
| Zn (μg/L) | 634.4 ± 114.4 | 723.3 ± 142.6 | 0.01, $t = -2.6$ |
| Cp (mg/L) | 353.6 ± 105.2 | 386.4 ± 135.0 | 0.29, $t = -1.05$ |
| Zn/Cu R | 0.71 (0.27) | 0.74 (0.27) | 0.55, $z = -0.5$ |

**Table 5** Correlation between biochemical parameters and trace elements with proteinuria, C3, C4 and anti-dsDNA serum values

| *Biochemicals* | *Proteinuria p(r)* | *C3* | *C4* | *Anti-dsDNA* |
|---|---|---|---|---|
| Serum Alb (g/dL) | 0.001 ($r_s = -0.4$) | 0.4 ($r_s = 0.08$) | 0.02 ($r_s = 0.2$) | 0.007 ($r_s = -0.3$) |
| Cu (μg/L) | 0.03 ($r_p = -0.2$) | 0.5 ($r_p = 0.07$) | 0.03 ($r_p = 0.05$) | 0.7 ($r_p = -0.03$) |
| Se (μg/L) | 0.03 ($r_p = 0.2$) | 0.1 ($r_p = 0.1$) | 0.08 ($r_p = 0.1$) | 0.4 ($r_p = 0.09$) |
| Zn (μg/L) | 0.001 ($r_p = -0.4$) | 0.4 ($r_p = 0.08$) | 0.6 ($r_p = 0.04$) | 0.1 ($r_p = -0.1$) |
| Cp (mg/L) | 0.3 ($r_p = -0.1$) | 0.3 ($r_p = 0.1$) | 0.3 ($r_p = 0.1$) | 0.9 ($r_p = 0.001$) |
| Zn/Cu R | 0.9 ($r_s = 0.004$) | 0.2 ($r_s = 0.1$) | 0.9 ($r_s = -0.06$) | 0.4 ($r_s = -0.08$) |

**Table 6** Cut-off point values for laboratory parameters, which showed lower concentrations in patients than healthy controls

| | *Cut-off value* | *Sensitivity* | *Specificity* | *(+LR)* | *(-LR)* | *(+PV)* | *(-PV)* |
|---|---|---|---|---|---|---|---|
| Serum Se (μg/L) | ≤109.3 | 75.6 | 75.6 | 3.09 | 0.32 | 75.6 | 75.6 |
| Alb (g/dL) | ≤4.2 | 40.48 | 91.75 | 4.94 | 0.65 | 81.0 | 64.0 |
| Zn (μg/L) | ≤800 | 83.72 | 68.04 | 2.62 | 0.24 | 69.9 | 82.5 |
| Zn/Cu R | ≤0.82 | 69.05 | 71.13 | 2.39 | 0.44 | 67.4 | 72.6 |

these trace elements and Alb with anti-dsDNA, C3 and C4 (Table 5).

*Correlation of trace elements with each other in lupus patients*

There was a significant correlation between serum values of the following parameters: Cu and Cp ($p < 0.001$, $r_s = 0.5$); Zn and Alb ($p = 0.008$, $r_s = 0.1$); Zn and Cu ($p = 0.008$, $r_s = -0.1$).

*Comparison of trace element serum values between patients under treatment with cytotoxic drugs or prednisone and who were not*

The correlations between serum values of Alb, Zn, Cu, Cp, Zn/Cu R, Se and average daily prednisolone dosage was evaluated. Only serum Cu ($p = 0.02$, $r = -0.39$) showed a negative correlation and Zn/Cu R ($p = 0.006$, $r = 0.46$) showed a positive correlation with the average daily prednisolone dosage. When patients were divided into two groups according to whether they were treated with cytotoxic drugs or were cytotoxic drug naïve patients, statistical analysis demonstrated that serum Alb ($p = 0.01$, $z = -2.4$) and Cu ($p = 0.01$, $t = -2.5$) values were significantly lower in patients under treatment with cytotoxics.

*MedCalc analysis*

There was a significant difference in the distribution of serum trace elements including Se, Alb, Zn and Zn/Cu R between patients and controls (Table 6). Serum values of all those parameters in the table were higher in healthy controls.

*Logistic regression analysis: influence of patients' demographics, trace elements and Alb on SLEDAI category*

Forward conditional logistic regression was used to assess the association between independent variables including age, disease duration, prednisolone dosage, serum values of Se, Cu, Zn, Cp, Zn/Cu R and Alb in patients and the dependent variable for the SLEDAI category (SLEDAI ≤4 and SLEDAI >4). Only serum Alb serum was associated with SLEDAI category ($p < 0.001$, $\beta(SE)$: $-1.3 \pm 0.34$).

*Logistic regression analysis: influence of participants' demographics and trace elements on lupus and the healthy group*

The forward conditional logistic regression analysis method was used to assess the association of independent variables including age, gender, serum values of Se, Cu, Zn, Cp, Zn/Cu R and Alb in the case and control groups; Alb: ($p < 0.04$, $\beta(SE)$: $-0.57 \pm 0.29$), Zn: ($p < 0.001$, $\beta(SE)$: $-0.009 \pm 0.002$) and Se: ($p < 0.001$, $\beta(SE)$: $-0.06 \pm -0.01$) were higher in the case group.

## Regression analysis for Se, Zn and Cu as dependent variables and other demographics and trace elements as independent variables

*Se*

In the primary analysis, we found serum Se was higher in healthy controls than for lupus patients.

We then considered serum Se as a dependent variable and analyzed the effect of age, gender and aforementioned groups (lupus patients and healthy controls) on it. It was again found that only the healthy individuals ($p < 0.001$, $\beta(SE)$: $26.3 \pm 2.8$) correlated with higher Se concentration. Gender ($p = 0.45$, $\beta(SE)$: $4.64 \pm 6.2$) and age ($p = 0.75$, $\beta(SE)$: $0.38 \pm 0.12$) showed no significant relationship with serum Se in regression analysis.

In analysis with Se as the dependent variable and SLEDAI, age, prednisolone dosage, disease duration and proteinuria as independent variables, we found that only proteinuria had significant influence on Se serum values: ($p = 0.025$, $\beta(SE)$: $3.4 \pm 1.3$) as was previously shown in the correlation analysis (Table 5).

*Cu*

There was a negative correlation between SLEDAI and serum Cu. With serum Cu as a dependent variable and SLEDAI, prednisolone dosage, age, sex and proteinuria as independent variables, we found by regression analysis that only SLEDAI remained significant in the model ($p = 0.04$, $\beta(SE) = -4.9 \pm 2.4$).

*Zn*

Serum Zn values were lower in SLE patients than healthy volunteers. Using regression analysis considering Zn as a dependent variable and age, sex, Alb and patient or control groups as independent variables, we found that Alb ($p < 0.001$, $\beta(SE) = 44.8 \pm 10.1$) and the healthy group ($p < 0.001$, $\beta(SE) = 126.7 \pm 20.0$) had a positive influence on Zn serum values.

In the next step, by regression analysis, Zn was selected as an dependent variable and SLEDAI, sex, age, disease duration, prednisolone dosage, proteinuria and serum Alb were chosen as independent variables; only Alb stayed in the model ($p < 0.001$, $\beta(SE) = 69.6 \pm 10.3$).

## Discussion

The main outcomes of the current study were that serum concentrations of Alb, Se and Zn/Cu R were lower in lupus patients compared with healthy age- and sex-matched controls. There was a negative correlation between serum Cu levels and SLEDAI2K. Additionally, serum concentrations of Cu and Zn were significantly lower in patients with overt proteinuria.

## Trace elements and lupus

Despite the likely role of peroxidative damage in autoimmune diseases, there have been relatively few studies investigating the relationship between trace elements and the disease activity of SLE; notably important ones are Yilmaz et al.[12]and Almroth et al.[15]

If oxidative stress is an intrinsic part of the process of cell damage in SLE, then anti-oxidative enzymes, for example superoxide dismutase and glutathione peroxidase, may protect cells. Superoxide dismutase is responsible for the removal of superoxide ions and requires Cu and Zn as cofactors.[16,17] Furthermore, glutathione peroxidase by enrolling Se elements activates the arachidonic acid cascade in cyclooxygenase and lipoxygenase pathways.[18] Some researchers observed that glutathione peroxidase as well as superoxide dismutase decline in active lupus.[19]

## Lupus and Alb values

Serum Alb is capable of transporting a small proportion of Cu ions and to inhibit lipid associated peroxidation and consequently to reduce hydroxyl-radical formation.[12] Furthermore, Alb binds to the majority of plasma Zn.[20] With respect to the acute phase response[21,22] and the main protein excreted from urine in proteinuric patients, it is predictable that our results were in line with other similar studies.[12] Moreover, logistic regression analysis showed that even after considering Alb, other serum trace element concentrations and demographic data as independent variables, serum Alb, Zn and Se concentrations remained higher in healthy group.

Assessment of disease activity in this study showed a significant negative correlation between Alb concentrations and lupus activity. This result has been shown in previous studies.[21,22] This is a consequence of lower Alb levels in active patients secondary to kidney damage and the acute phase response, affecting Alb. Another important finding of the current study was the positive correlation between Alb and Zn. Bates et al.[23] demonstrated that the concentration of serum transport proteins like Alb were depressed in patients with Zn deficiency and levels of those proteins corrected with Zn supplementation. As mentioned above, positive correlation between Zn and Alb may stem from the Alb binding capacity of Zn.

## Lupus and Zn values

Compared to the control group, Zn values were lower in patients with SLE, which was confirmed following logistic regression analysis. Yilmaz et al.[12] have reported a similar result. However, Almroth et al.[15] did not observe a significant difference in serum Zn concentrations between patients with SLE and healthy volunteers. Zn and Cu are cofactors for superoxide dismutase-1, and serum Zn may fall in SLE; this may be explained by an increased Zn consumption due to oxidative stress[24–28] or conversely low Zn levels in this situation may lead to low anti-oxidative activity and cell damage. Some studies have also shown that serum superoxide dismutase concentrations fall in active SLE and may be prevented by treatment with omega-3 fatty acids.[29] Some studies pointed to the protective role of Zn in the development of rheumatoid arthritis.[30] Importantly, profound Zn deficiency may lead to thymus atrophy and T-lymphocyte dysfunction.[31] Thus, Zn deficiency may serve a potential role in T cell function that disregulates in SLE.

Although, we did not find any correlation between serum Zn values and disease activity in SLE, an association between serum Zn and the amount of urine protein excretion was found which might hypothesize an indirect reflection of losing Zn from the impaired kidney or diversely its active function in the induction of kidney damage. However, regression analysis of Zn as an dependent variable and other trace elements and disease specific manifestations as independent variables showed that when Alb and the degree of proteinuria were entered into the model, only Alb remained as being significant because of strong correlation between Alb and proteinuria and stronger correlation between Alb and Zn than correlation between Alb and the quantity of proteinuria. It should be mention that abnormalities of Zn metabolism and status secondary to increased urinary or endogenous Zn excretion have been well established in nephrotic syndrome and kidney damage. Previous studies reported that low serum values of Zn in nephrotic syndrome are not due to excretion of Zn bounded to urine proteins. Animal studies have suggested that Zn deficiency in proteinuria may be a result of increased serum and urine levels of certain amino acids (cysteine, histidine) that are associated with greatly increased urinary Zn excretions.[32]

Results of a few studies on Zn levels in nephrotic syndrome, or other reasons for proteinuria, demonstrated different results, from equivalent Zn values in proteinuric patients in comparison to healthy individuals to lower Zn levels in the first group. However, correlation of Zn and urine protein levels was not mentioned in those surveys.[33–35]

Finally, Zn has a critical function in diverse body activities including wound healing, reproduction, metabolic homeostasis, neutrophil and lymphocyte activities; growth in children and optimal neural activities, indicates that more studies on the probable alleviating function of Zn in lupus are required.[36]

## SLE and serum Cu and Cp

Serum Cu levels were found to be negatively correlated to disease activity, cytotoxic therapy and prednisolone dosage in SLE. Moreover, serum Cu concentrations were negatively correlated with proteinuria. However, regression analysis showed that of the demographic factors assessed, and using the above variables as independent factors and Cu as a dependent variable, only SLEDAI remained significant in the model. Serum Cu and Cp concentrations were not different between the SLE and healthy groups. Data obtained from Yilmaz et al.[12] and Lipin et al.[37] showed higher values of serum Cu and Cp in SLE patients than healthy controls, and Yilmaz et al.[12] found a positive correlation between serum Cu concentrations and SLEDAI.

A positive correlation between serum Cu and Cp was found for the whole group of participants. Cu ions may be involved in the production of reactive oxidative species (ROS), and the consequent damage to DNA and other bio-molecules. On the other hand, Cp has anti-oxidative properties. However, despite the above information, there is some evidence that excessive Cu intake is not an important factor in the development of general disease state.[38] A study by Duffy et al.[39] illustrated that Cu supplementation does not improve disease activity in SLE. Since Cp is an acute phase reactant; it may be expected that its concentrations are raised in SLE, but due to proteinuria or other unknown mechanisms that prevents from elevation of other inflammatory markers like CRP[40] in lupus. This marker presented no change in lupus patients in this study. Immuno-suppressive and immuno-modulatory drugs may be responsible for these results as well.

## Lupus and Zn/Cu R

The serum Zn/Cu R was found to be lower in SLE patients than in the controls in this study. However, we found no correlation between this parameter

and disease activity. The Zn/Cu R is a good marker for malnutrition. It has been proposed that lupus patients may suffer from subtle malnutrition due to gastrointestinal involvement, proteinuria and drug side effects.[41] In addition, Zn-Cu superoxide dismutase (SOD) is an anti-oxidative enzyme. Serum Zn and Cu values have previously been shown to be inversely related; in other words, it has been observed that excessive Cu intake may reduce Zn values and vice versa. Therefore, the balance between those two trace elements is important for maintaining normal biochemical functions.[42] There are no data available about Zn/Cu R in SLE. Although lower values were predictable in our research for secondary to lower serum Zn values in patients, this ratio should be considered separately in autoimmune diseases. The Zn/Cu R is an indicator of malnutrition. As subclinical malabsorption can develop in lupus, it may be important to evaluate patients with lower Zn/Cu R for this problem.[43] This study also showed a positive correlation between prednisolone dosage and Zn/Cu R probably secondary to a negative correlation between prednisolone and Cu serum values.

## SLE and Se values

Se levels in lupus patients were lower compared to the control group, which was supported by regression analysis. Almroth et al.[15]have also reported the same results. Serum Se has been reported to be lower in rheumatoid arthritis than for healthy volunteers.[44–45] There is some evidence that, for countries in which rice is the main source of nutrition, like Iran, Se deficiency is not prevalent.[46] Se is the main constituent of glutathione peroxidase, as is the same for superoxide dismutase, and plays a crucial role in the antioxidant defense system. Therefore, a decrease in glutathione peroxidase activity in lupus patients might be the cause of the lower amount of serum Se. Se is one of the important trace elements; its concentration varies with soil type and climate. Se as a main part of seleno-proteins serves an important role in reproductive functions, thyroid hormone synthesis and immune-modulation via adaptation of T helper cells, natural killer cells and antibody formation.[47] Additionally, Fatmi et al.[48] showed that in diabetic rats with dietary induced Zn deficiency, Se supplementation can compensate for some features of the Zn insufficiency.

A study of Iran projects found that although soil Se level in different parts of the country is low, its mean value in essential crops (rice, wheat, date, etc.) is high enough.[46] Accordingly, Se deficiency in lupus patients is not secondary to geographical condition or dietary habits. In this research, a positive correlation by correlation test and regression analysis was found between proteinuria and serum Se values. It was in contrast to the results of a few studies on this purpose that demonstrated the protective role of Se against glomerular and tubular damage in kidney.[49–50]

## Study limitations

In this study, measurements of serum trace elements have been done at a single time point which may not necessarily indicate overall trace element status. Moreover, as it was a cross-sectional study, disease activity was also computed at a single time point. To evaluate the influence of fluctuations in disease activity on serum trace elements and vice versa, longitudinal studies might be definitely more helpful. Furthermore, treatment with drugs such as steroids or cytotoxics may confound results. It is obvious that it was not possible to discontinue treatment strategies for a period prior to sampling.

## Conclusion

A significant decrease in serum Alb, Se and Zn as well as Zn/Cu R was observed in lupus patients compared with healthy age- and sex-matched healthy volunteers. Moreover, there was a negative correlation between disease activity and serum Alb and Cu levels. Since we carried out a cross-sectional study, the results cannot be used to impugn the role of trace elements in SLE status. More detailed studies are required to find out if supplementation with those trace elements, geo-medicine strategies or nutritional habits can help to alleviate lupus manifestations or not.

## Funding

This article was extracted from the thesis prepared by Mr Masoud Abrishami-Moghadam to fulfill the requirements for earning the Internal Medicine Specialty Degree. The Research Council of Mashhad University of Medical Sciences, Mashhad, Iran is appreciated for financially supporting this study, grant number (89292).

## Conflict of interest statement

The authors had no conflict of interest.

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