Selenium, copper, zinc, iron levels and mortality in patients with sepsis and systemic inflammatory response syndrome in Western Black Sea Region, Turkey

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ORIGINAL ARTICLE

Selenium, copper, zinc, iron levels and mortality in patients with sepsis and systemic inflammatory response syndrome in Western Black Sea Region, Turkey

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Abstract

Objective: To evaluate the changing levels of selenium, copper, zinc and iron in patients with sepsis and systemic inflammatory response syndrome and their influence on mortality.

Methods: The prospective study was conducted at a tertiary care university hospital in Zonguldak city in the western Black Sea region of Turkey from January 2012 to December 2013, and comprised patients with sepsis and systemic inflammatory response syndrome. Blood samples were taken on 1st, 3rd, 5th and 7th days to measure serum selenium, copper, zinc and iron levels. Patients' demographic data, presence of additional diseases and mortality were recorded.

Results: Of the 57 patients, 28(49.1%) were female and 29(50.9%) were male, with an overall mean age of 60.3±19.4 years, mean height of 166.1±11.4cm, mean weight of 76.5±17.5kg. Copper and zinc levels were in the normal range, while selenium and iron levels were lower than the limit values at all measuring periods. There was no significant difference between first and other days in accordance with element levels ($p>0.05$). Baseline copper levels in patients with malignancy were lower than patients without malignancy ($p<0.05$). In hypertensive patients, baseline copper levels were higher and 7th day levels were lower than non-hypertensive ($p<0.05$). Baseline selenium levels of those who died were lower than the other patients ($p<0.05$). Selenium and iron levels were decreased in patients with sepsis-systemic inflammatory response syndrome and copper levels were lower in patients with malignancy, hypertension and chronic obstructive pulmonary disease ($p<0.05$). There was no change in zinc levels of the patients.

Conclusion: Reduced basal selenium levels of patients with sepsis and systemic inflammatory response syndrome were associated with mortality.

Keywords: Copper, Iron, Selenium, Sepsis, SIRS, Zinc. (JPMA 66: 447; 2016)

Introduction

Oxidative injury plays a significant role in the development of sepsis and systemic inflammatory response syndrome (SIRS). In anti-oxidant defence system, trace elements such as selenium (Se), copper (Cu), zinc (Zn) and iron (Fe) act as the key to prevent oxidative damage by scavenging oxygen free radicals. It is known that plasma Se deficiency may be common in critically ill patients and associated with higher mortality in European xountries.[1] Low plasma Se concentrations may be associated with depletion of Se in soil and low intake of Se also leads to systemic inflammation.[2,3] Fe deficiency is the most prevalent nutritional deficiency. Also, Fe treatment has been related to mortality and morbidity in critical patients. Zn levels are associated with the development of multiple organ failure in patients with sepsis.[4] Low serum levels of Zn and Cu have recently been reported in association with total parenteral nutrition and fasting state.[5] Cu levels are correlated with Zn and acute inflammation.[6] To date, trace element supplementation remains controversial, but has demonstrated significant benefit for patients with sepsis and for the prevention of several infectious diseases.[7] The Black Sea region is a mountainous region which has been known to suffer from some of trace element deficiency and it is also a centre of endemic goitre in Turkey.[8] There are no studies in Turkey assessing the influence of region on trace elements plasma concentrations in patients with sepsis and SIRS.

The current study was planned to evaluate the change in the levels of Se, Cu, Zn and Fe of patients with sepsis and SIRS, and their influence with mortality.

Subjects and Methods

The prospective study was conducted at a tertiary care university hospital in Zonguldak city in the western Black Sea region of Turkey from January 2012 to December 2013, and comprised patients with sepsis and SIRS. The study was approved by the ethics committee of Bülent

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Ecevit University and written informed consent was obtained from the patients and their relatives.

Sepsis and SIRS were defined by the International Sepsis Definitions Conference.[9] After the diagnosis of sepsis and SIRS, blood samples were taken on 1st (baseline), 3rd, 5th and 7th days to measure serum Se, Cu, Zn and Fe levels. Patients were treated according to the recommendations in sepsis guideline.[9] All patients received nutritional support via enteral, parenteral or mixture nutrition within 24-48 hour and after resuscitation for haemodynamic stability but there were no additional trace element supplementation. Patients were considered haemodynamically stable if they were not hypotensive and did not require high-dose catecholamine, large volume fluid or blood product resuscitation.[10] Energy requirements were calculated according to the predicted basal metabolic rate (via Harris Benedict formula), initially 50% of the total volume.[11] The infusion rate increased every 12 to 24 hours, if tolerated to reach 100% of total estimated volume by day 2.[12]

Patients' demographic data, smoking and alcohol usage, meal types, presence of additional diseases (trauma, cardiac disease[CD], hypertension [HT], malignancy, operation history, chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), renal failure [RF] and goitre) and patients' 28-day mortality were recorded. Trace elements were analysed using atomic absorption spectrometry, ferrozine method, and calorimetric method.

In terms of measurements of trace elements,[13] serum Cu and Zn levels were measured by using commercial kits (FAR-SRL, Verona, Italy) in ultraviolet (UV)-1601 spectrophotometer device (Shimadzu, Tokyo, Japan) with colorimetric method. The reference range for Cu was 70-155 µg dL-1 and for Zn 70-120 µg dL-1.

Selenium was measured using Perkin Elmer branded Atomic Absorption Spectrometer (AAS), and hydride system (FIAS). One unit serum + 6 unit acid solution (per-chloric/nitric acid solution, 5/1) was hydrolysed at 120°C for 1h. A total of 3mL 50% hydrochloric (HCl) acid was added, and was hydrolysed at120°C for 1h again. Then 3mL water was added and the last reading was done. Five-point calibration was performed using Inorganic Ventures branded stock. Two levels were used as controls, such as Serenom Trace Elements Serum L-1 (selenium level 100-114 µg L-1) and Serenom Trace Elements Serum L-1 (selenium level 153-173 µg L-1). The reference range was 46-143 µg L-1 for serum selenium level in the laboratory that the measurement was performed.

Fe measurement was carried out using the Ferrozine method[14] according to which, iron was released from transferrin under acidic conditions. It was reduced to ferrous form, and was coupled with a chromogen for colorimetric measurement. In this procedure, iron was measured directly without any steps of protein denaturation or any interactions with an endogenous copper. Ferric iron was separated from the carrier protein transferrin in acidic condition, and was reduced simultaneously to ferrous form. Then iron formed a complex with ferrozine, a sensitive iron indicator, and formed a coloured chromophore that exhibited absorbance at 571/658 nm.

Reference values used were: male, 65-175 µg dL^{-1} (11.6-31.3 µmol L^{-1}); female, 50-170 µg dL^{-1} (9.0-30.4 µmol L^{-1}); transferrin (Fe^{3+}) → Apotransferrin + Fe^{3+}; Fe^{+3} + Ascorbic Acid → Fe^{+2}; Fe^{+2} Ferrozin → Fe^{+2} + Ferrozin complex.

Data was analysed using SPSS 16. Results were expressed as either mean ± standard deviation (SD) or as frequencies and percentages. Mann-Whitney U test was used to compare serum element levels between groups of those having additional disease or not. Paired samples test and Wilcoxon test were used to compare serum element levels between consecutive measurements (1st, 3rd, 5th, 7th days) in the same group. Results of analysis were evaluated with 95% confidence interval (CI), and p<0.05 values were accepted as statistically significant.

Results

A total of 70 patients were initially enrolled, but 13(18.5%) with severe sepsis and septic shock died in 7 days after the

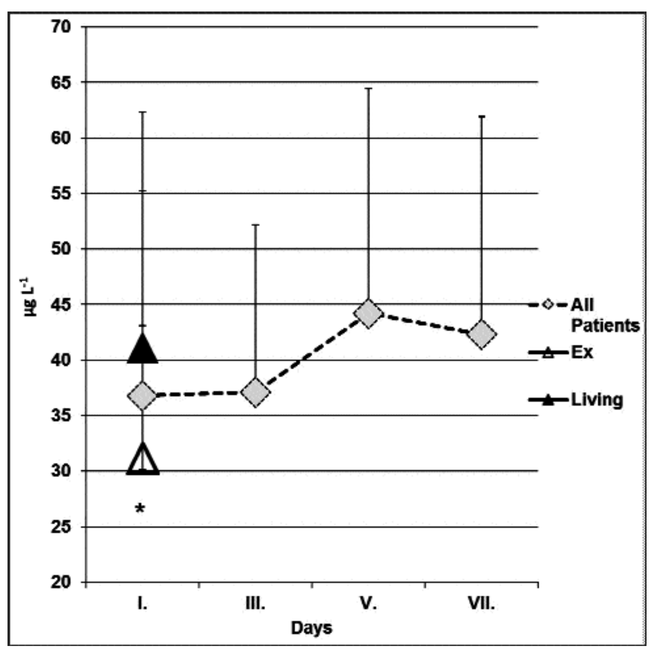

Figure-1: Serum (Se) levels (µg.L^{-1}). *p<0.05 Surviving (living) patients Se levels compared with non-surviving (ex).

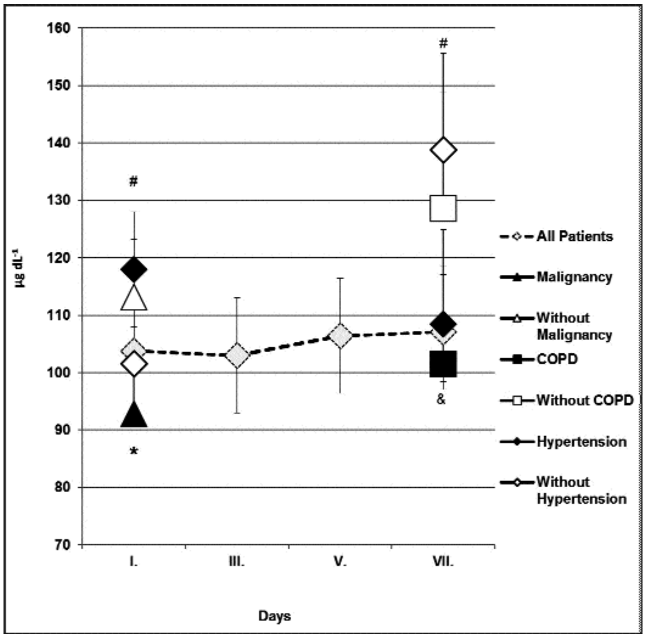

Figure-2 Serum Copper (Cu) levels (μg.dL^{-1}); *p<0.05 Patients with malignancy compared with without malignancy; #p<0.05 Patients with hypertension compared with without hypertension; and p<0.05 Patients with chronic obstructive pulmonary disease (COPD) compared with those without COPD.

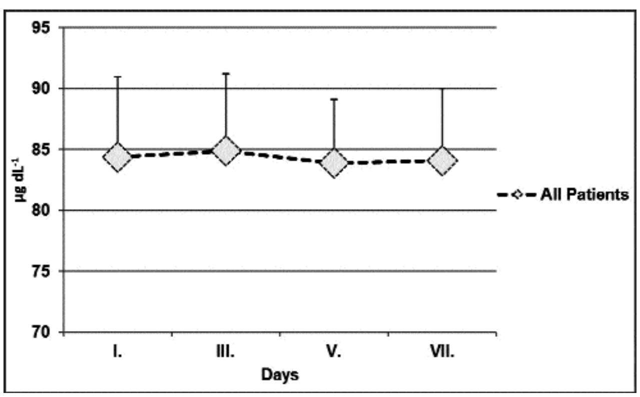

Figure-3: Serum zinc (Zn) levels (μg.dL^{-1}).

diagnosis despite standard treatment protocol and were excluded. The study was completed with 57(81.4%) patients. Of them 28 (49.1%) were female and 29 (50.9%) were male, with an overall mean age of 60.3±19.4 years, mean height of 166.1±11.4cm, and mean weight of 76.5±17.5kg. There was no vegetarian patient. There were 9(15.8%) patients with history of smoking, 2(3.5%) of alcohol consumption. There were 4(7%) trauma, 18(31.6%) CD, 13 (22.8%) HT, 4 (7%) malignancy, 7 (12.3%) operation, 11 (19.3%) COPD, 9 (15.8%) DM, 5 (8.8%) RF and 25 (43.9%) exitus cases. There was no goitre patient.

Table: First-day elements levels of patients with additional disease and mortality.

	Fe (μg.dL^{-1})	Cu (μg.dL^{-1})	Zn (μg.dL^{-1})	Se (μg.L^{-1})
Trauma -	26.4±2.2	111.4±21.0	86.7±7.4	33.0±12.6
Trauma +	18.9±6.3	103.5±3.1	85.8±7.3	36.2±19.5
p	0.376	0.486	0.727	0.844
CD -	26.9±2.2	102.9±16.6	86.7±9.0	31.2±11.0
CD +	24.5±10.9	114.9±204	86.4±6.4	34.7±14.7
p	0.225	0.087	0.718	0.811
HT -	26.2±26.6	101.06±16.2	88.1±6.9	31.7±12.2
HT+	25.2±11.6	118.0±18.7	84.2±7.6	36.1±14.9
p	0.279	0.013	0.180	0.467
Malignancy -	27.0±21.5	113.2±19.5	85.8±7.5	35.0±16.6
Malignancy +	15.2±4.3	92.8±8.3	91.3±3.8	38.7±20.2
P	0.067	0.043	0.153	0.241
Operation-	26.2±22.7	109.0±19.3	87.1±7.9	34.3±12.8
Operation +	22.8±11.0	114.7±21.7	84.9±5.3	30.9±15.8
p	0.799	0.524	0.331	0.353
COPD -	26.3±24.8	106.2±12.9	85.8±7.5	35.7±15.5
COPD +	24.9±13.0	113.9±25.9	87.1±7.4	30.6±8.9
p	0.621	0.525	0.706	0.688
DM-	27.1±2.4	108.2±18.6	86.7±6.6	31.7±14.7
DM+	22.8±10.1	111.4±20.9	85.4±9.0	38.0±10.2
p	0.980	0.676	0.941	0.051
RF-	25.9±22.3	109.1±16.5	87.6±6.7	34.0±14.1
RF+	24.8±12.0	109.8±30.7	80.6±8.2	32.8±11.4
p	0.653	0.719	0.071	0.950
Alive	23.6±12.3	121.0±15.8	89.2±7.3	43.7±12.0
Dead	37.4±45.7	109.8±20.2	91.8±3.3	27.1±2.6
p	0.903	0.110	0.536	0.016

-: Absent +: Present, CD: Cardiac disease, HT: Hypertension, COPD: Chronic obstructive pulmonary disease, DM: Diabetes Mellitus, RF: Renal Failure.

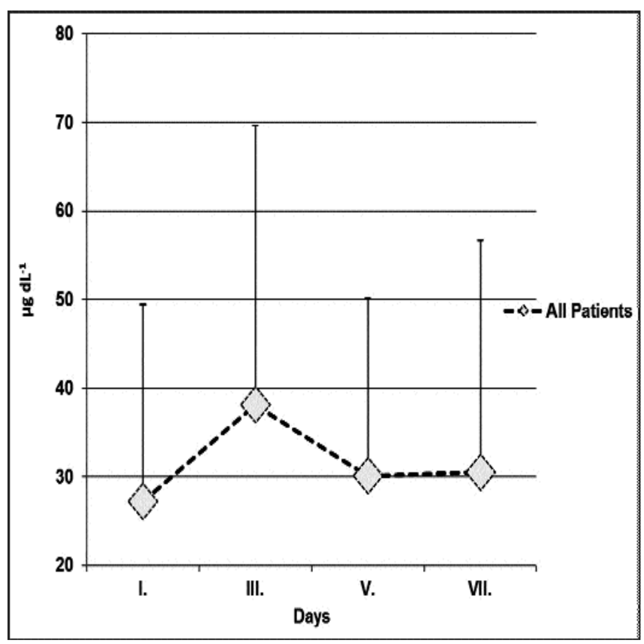

Figure-4: Serum iron (Fe) levels (μg.dL^{-1}).

First-day element levels of patients with additional disease and mortality recorded (Table). Although Cu and Zn levels were in normal range, but Se and Fe levels were lower than the limit values at all measuring periods. There were no significant differences between first and other days for all element levels ($p>0.05$) (Figures 1-4). Baseline Cu levels in patients with malignancy were lower than patients without malignancy ($p<0.05$).. In HT patients, baseline Cu levels were higher ($p<0.05$) and 7th day Cu levels were lower than non-HT patients ($p<0.05$). Similarly, Cu levels on 7th day of patients with COPD were lower than patients without COPD ($p<0.05$). Baseline Se levels of those who died were lower than the rest ($p<0.05$). There were no significant differences between groups having additional diseases or not in the element levels ($p>0.05$ each).

Discussion

This is the first clinical trace elements trial in patients with sepsis and SIRS in the western Black Sea region. It was found that Se and Fe levels were decreased in patients with sepsis and SIRS as a result of increased oxidative stress and use of anti-oxidant systems. Cu levels were lower in patients with malignancy, HT and COPD in sepsis and SIRS in the study region of Turkey. There were no change in Zn levels of patients with sepsis and SIRS. It was concluded that reduced baseline selenium levels were associated with mortality in sepsis and SIRS.

It was shown that oxidative stress changes trace elements levels.[13] Trace element levels in critical patients were decreased by factors such as increased oxidative stress, use of anti-oxidant sytems, haemodilution with fluid resusciation, renal replacement therapies, losses in biological fluids and inadequate intake.[15] Systemic inflammation affected oxidative stress in sepsis and SIRS. Iron, selenium, copper and zinc are required for the activity of antioxidant enzymes. Low-trace elements are associated with higher risk of death, SIRS, multiple organ failure (MOF) and higher oxidative stress in critically ill patients.[15] It is well established that Se plays an important role in the anti-oxidant defence systems and protection against lipid peroxidation and thyroid hormone metabolism.[3,16] Lower plasma Se levels associated with oxidative stress, organ dysfunction and higher mortality in European trials were attributed to the depletion of selenium in soil and food.[17]

Zonguldak is a city in the western Black Sea region of Turkey, and it rains the whole year and its residents make their living from coal mining. Also, COPD is frequently seen in Zongudak because of air pollution. Black Sea region is a mountaineous region which has been known to suffer from iodine deficiency and a centre of endemic goitre in Turkey.[8] In our study there was no patient with goitre but all patients suffered from selenium deficiency.

In previous trials, similar to the present study it was shown that plasma selenium concentration decreased in early acute response in sepsis.[18] In our study we found that basal Se levels were lower than reference values (<46-143 µg.L-1). Se is an essential micronutrient for anti-oxidant defences. It was shown that Se status declines with sepsis disease, especially in non-survivors.[19] Meta-analyses have shown that Se might have beneficial effects on patients with sepsis and SIRS.[20] The optimum dose and efficacy of Se still remain controversial. Correlation between plasma selenium and mortality were found in our study.

Worlwide iron deficiency is the most prevelant nutritional deficiency. Iron is a critical factor for survival and virulance of pathogenic microorganisms. It was known that Fe requirements increase during infections and increased oxidative stress might play a role in the pathogenesis of iron deficiency. Also iron treatment has been associated with acute exacerbations of infection and sepsis.[21] Our study found that Fe levels of patients were decreased, and monitoring of serum Fe levels may be useful in patients with sepsis and SIRS.

Zn is an essential trace element, co-factor of Copper-Zinc superoxide dismutase (SOD) enzyme, plays an important role for host response to infection. In SOD enzyme, Zn causes the enzyme to keep stability, and Cu is responsible for the activity of the enzyme.[22] Clinical researches have reported that Zn deficiency correlates with an increased severity of illness and progress of MOF in patients with sepsis.[4] Zn supplementations is still controversial. There is no recommendation for routine Zn supplementation in critically ill patients. Some of studies have concluded that low plasma Zn levels are usually misinterpreted for Zn deficiency and Zn is unnecessarily supplemented.[23] A previous controlled trial reported that Zn reduced treatment failure in infants with serious bacterial infection.[24] Supplementation of Zn needs to be considered with Cu replacement due to interference with Cu absorption.[25] Also, it was reported that an increased Cu/Zn ratio might indicate an impaired anti-oxidant defence.[22] In our study serum Zn levels were in the normal range.

Copper is important for collagen synthesis, antioxidant activity, iron transport and can cause anaemia, leukopenia and pancytopenia also acts as a cofactor for oxidative metalloenzymes.[26] Cu deficits can contribute to the development and progression of cardiovascular disease (HT) and DM.[27] Cu has significant effects on lipid peroxidation. Also, it was reported that myocardial infarction (MI) risk was four times more in humans with high plasma Cu levels in comparison with the normal population as a result of negative effects of lipid peroxidation on the vessel walls.[28] We found that in hypertensive patients, basal Cu levels were higher and 7th day Cu levels were lower than non-hypertensive patients. Copper in the vessel wall that causes lipid peroxidation may explain higher basal Cu levels in our hypertensive patients. In patients with cancer, it was stated that serum Cu/Zn ratio increased significantly in the advanced stages.[29] In the present study, basal Cu levels in patients with malignancy were lower than patients without malignancy. It was reported that serum copper levels increased in COPD.[30] In our study, Cu levels on 7th day of patients with COPD were lower than patients without COPD. On the contrary of previous study, it was seen that Cu levels reduced in the presence of co-morbid diseases such as COPD, HT, malignancy which can increase the oxidative stress in sepsis and SIRS.

The main limitation of this study was that we did not know patients trace elements levels untill finishing study, because if we knew the patients' Se status in the early period, Se replacement might have had beneficial effects.

Conclusions

Low plasma Se concentration can be associated with mortality of sepsis and SIRS. Trace element levels should be monitored not only in sepsis and SIRS cases, but also in regional soil for well-qualified management of these cases and if it is necessary, patients with low trace element level should be supported with an appropriate nutrition plan.

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