Trace elements (Se, Zn, and Cu) levels in patients with newly diagnosed acute leukemia

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Abstract

Objective: This study was conducted to evaluate serum trace elements level, selenium (Se), zinc (Zn), and copper (Cu) at the diagnosis of acute leukemia, and also if there is any difference in their serum levels among various subtypes of disease.

Subjects: Fifty newly-diagnosed acute leukemia patients before starting therapy and fifty healthy subjects were entered into the study.

Methods: Serum trace elements level was determined by atomic absorption spectrometry technique.

Results: Serum selenium and zinc levels were significantly lower in patients than in healthy subjects. The comparison of trace element levels in acute myeloid leukemia (AML) subtypes showed significant difference in Se levels between AML-M3 and AML-M5. There were no significant difference between Cu levels of patients and healthy subjects.

Conclusions: Low serum levels of Zn and Se in leukemia patients in this study can express the need for assessing the effect of improving trace elements status on patient`s overall survival. A difference in Se levels between subgroups of AML patients warrants further investigation with larger sample size.

Keywords: Acute leukemia, Antioxidants, Trace elements, Selenium, Zinc, Copper

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Introduction

Leukemia is one of the most common cancers in adults. According to the etiology of neoplastic disease and alteration in antioxidants levels, many researches over recent years have studied if trace elements have any modifying effect in clinical outcome. Trace elements have some fundamental roles within human body like protection against cellular oxidative stress and synthesis and structural stabilization of proteins and nucleic acids.1[1]

Selenium (Se) is known as a cofactor of selenoproteins such as glutathione peroxidases (GSH-Px) a family of antioxidant enzymes.[2, 3] Se intakes above those needed to maximize selenoproteins has an anti-cancer effect and increase production of cytotoxic T-cells and natural killer cells [4]. Increased Se levels have been shown to lower cancer mortality in studies.[5, 6] Se is present in red blood cells and various blood proteins like haemoglobin and albumin.[4, 7] Acute severe illnesses can alter bioavailability and requirement of trace elements through decrease absorption, increase urinary loss and alteration in element binding proteins .[8]

Zn and Copper(Cu) are cofactors of the antioxidant enzyme, superoxide dismutase (Cu/Zn-SOD) which detoxifies the toxic superoxide radical.[9-11] It is proven that measuring serum Cu level could be useful in assessing disease activity and response to treatment in some kinds of lymphoma and acute leukemia.[12]

The role of trace elements and their association with cancer can be very difficult to determine due to the large variation in concentrations of trace elements within the population and in normal ranges.[13, 14]

In a previous study, Hadjibabaie et al. identified low levels of Se in almost all the patients who underwent bone marrow transplantation.[15] This study was undertaken to evaluate the levels of Se, Zn and Cu in patients with newly diagnosed acute leukemia before receiving induction therapy.

Patients and Methods

Fifty patients (29 males and 21 females) who were newly diagnosed with acute leukemia from May 2010 to March 2011 were enrolled in the study. The patients were referred to and treated at Hematology-Oncology and Bone Marrow Transplantation Research Center, Tehran University of Medical Sciences (Shariati Hospital). None of the patients had previously been treated. Twenty-nine patients were male and twenty-one were female. The age ranged from 16 to 75 years (mean, 33.54±15.79). Twenty-eight of the patients (14 males, and 14 females) were affected by acute myeloid leukemia (AML) and 22 (15 males, and 7 females) by ALL. The control group consisted of 50 healthy subjects (25 males, and 25 females) from the personnel of the hospital. Their age ranged from 18 to 70 (mean 35.5±14.84). Both the study and control groups were almost of same socio-economic status with similar diet habits. AML patients were classified according to the French-American-British classification into M1 (n= 1), M2 (n= 7), M3 (n= 4), M4 (n= 10), and M5 (n= 6). Neither of the subject groups had taken vitamin and/or mineral supplements for at least six mounts before the study. The patients were subjected to one measurement of serum Se, Zn, and Cu before the start of the therapy. Signed informed consent was obtained from all participants at the beginning of the study.

Sample collection and storage

Venous blood was drawn in the morning after an overnight fast. Blood was collected via venipuncture and immediately centrifuged at 3000× g for five minutes. Two serum aliquots of 1.5 ml from each patient were stored in plastic tubes at -80 °C until the time of assay.

Determination of serum elements

Serum Se was determined by the graphite furnace atomic absorption spectrometry technique (Spectra AA 220, GTA 110, Varian, Australia) equipped with deuterium background correction Determinations were made in duplicate and the analyst was blinded to the status of the specimens.

In order to exclude the possibility of contamination with trace elements, all glassware and bottles were previously soaked in diluted nitric acid (10%) and rinsed with deionized water. The samples were diluted with 0.1% v/v triton X-100. We used a mixture of Pd + Mg(NO3)2 as matrix modifier in graphite furnace atomic absorption spectrometry with an appropriate furnace program (pyrolysis temperature of 900 °C and atom inducing temperature of 2600 °C).[16] The manufacturer's recommendations for wave-length, spectral band width and lamp current parameters were followed.

For determination of Zn and Cu, the serum samples were diluted five times with hydrochloric acid (0.1 N) for Zn and Cu measurements. A flame atomic absorption spectrometer (Spectra AA 220, Varian, Australia) equipped with deuterium background correction was used to measure the level of these elements. The Cu/Zn ratio (CZR) was calculated using the actual Cu and Zn values.

Detection of clinical parameters

The following hematological parameters were also determined in several patients: red blood cells (RBCs), hemoglobin (Hgb), and serum levels of albumin.

Statistical analysis

The results are shown as the mean±SD. The mean serum concentrations of Se, Zn, and Cu have been compared between patients and healthy subjects using the independent-samples t test. As the values of trace elements in patients' subgroups were not normally distributed and due to small sample size in some cases, Kruskal-Wallis test was performed in order to compare the levels of trace elements between different types of AML patients. Pairwise comparisons were done by Dunn procedure. The correlation of quantitative variables was assessed by calculating Pearson correlation coefficient. A p-value of <0.05 was considered as statistically significant.

Results

Selenium

This study included 50 patients compared with the control group. The difference in serum levels of Se between two groups of patients and controls was significant (p<0.05, Table 1). Se concentrations were higher in males than females in control group and age was not significantly correlated with serum Se either in healthy subjects or patients (11.48±1.58 in males and 10.32±1.42 in females, p=0.009).
The difference of Se levels between the two groups of AML and ALL patients was not significant (p=0.08).

Serum concentrations of Se in different subtypes of acute leukemia are shown in Table 2. There was only one patient in AML-M1 subgroup, so we did not mention that in the analysis. The difference in serum Se concentrations between subjects with AML-M3 and AML-M5 was statistically significant (P=0.02). The Se levels were significantly lower in patients with AML-M5.
Patients with acute leukemia had Se levels positively correlated with RBC (P=0.02) and Hgb (P=0.03, Pearson correlation was 0.31 and 0.29, respectively). Even though, the correlation of Se levels with albumin was not significant (P=0.07), but albumin levels were determined in only 18 patients (the mean albumin levels was 3.91±0.68).

Table 1. Serum zinc, copper, and selenium levels in healthy subjects and in patients with acute leukemia

		Control subjects	Acute leukemia	P-value
Mean±SD (μg/dl)	Se	10.90±1.60	7.23±2.21	<0.001
	Zn	98.59±17.88	49.00±15.91	<0.001
	Cu	86.15±14.56	84.58±17.96	0.65
	Cu/Zn	0.90±0.23	1.85±0.53	<0.001

Table 2. Serum zinc, copper, and selenium levels in different subtypes of acute leukemia

Disease		Mean±SD(μg/dl)		
		Se	Zn	Cu
AML	M2	8.35±2.41	41.80±12.87	71.60±18.51
	M3	9.95±1.88	47.66±12.66	77.33±6.35
	M4	7.04±2.31	59.87±19.46	91.87±20.91
	M5	5.56±1.71	40.60±21.98	76.80±10.70
	Total	7.48±2.49	49.23±18.88	81.38±18.00

Zinc

Zn levels were measured in 44 healthy subjects and 41 patients with acute leukemia, and the levels were significantly lower in patients group (p<0.001, table 1). The difference in Zn concentration between men and women was statistically significant in patients (p=0.03), but not in healthy subjects. The difference of Zn levels between two groups of AML and ALL patients and also in AML subgroups were not significant (p=0.09). Age was significantly correlated with serum Zn in patients (p=0.005), but not in healthy subjects (p=0.90).

Copper

There was no significant difference in levels of this trace element between healthy subjects and patients (table 1). Age was significantly correlated with serum Cu in patients ($p=0.005$), but not in healthy subjects ($p=0.07$). There was a positive correlation between serum level of Zn and Cu ($p<0.001$). The CZR was elevated in patients (1.85 ± 0.53) as compared with healthy subjects (0.90 ± 0.23, $p<0.001$).

Discussion

Trace elements, such as Se and Zn play a role in the protection against oxidative stress in cells. Oxidative stress produces free radicals which are involved in the pathogenesis of many diseases including hematological malignancy.[17]

Cancer-protective effect of selenium appears to be at serum concentration above 10.6 µg/dl in studies.[4] The mean serum Se level of healthy individuals in our study was similar to one reported in Tehran by Safaralizadeh et al. (10.90 ± 1.6 vs 10.06 ± 1.3 µg/dl) and some countries like Saudi Arabia (10.25 µg/dl) and the U.S. (11.02 µg/dl).[18]

There are several reports regarding decreased serum Se levels in patients with leukemia.[11, 19, 20] In this study, patients with acute leukemia (AML and ALL) showed a reduction in serum Se when compared with healthy subjects.

Acute myelogenous leukemia (AML) has several subtypes that differ in treatment and prognosis. A substantial difference in Se concentration was observed between subgroups of AML patients. The Se level was lower in AML-M5 as compared with AML-M3, showing that patients with AML-M5 are at higher risk for Se deficiency in comparison with other subtypes. The acute promyelocytic leukemia (AML-M3) is treated differently from all other subtypes and has the best prognosis among all types of AML. In contrast, among all types of AML, AML-M5 or monocytic leukemia that may infiltrate the skin and gums has a worse prognosis than other subtypes. To the best of our knowledge, no other studies have analyzed a possible difference in serum Se levels between different subgroups of acute myelogenous leukemia patients.

One explanation for our findings arises from the fact that rapid growth and enhanced uptake of Se by neoplastic tissue could be responsible for decreasing the trace element content of the blood which can lower serum Se concentration.

In our study, there was a significant gender effect on the Se level of healthy subjects. The mean level of Se in males was higher than females which could be as a result of selenium contribution in spermatogenesis. These findings are in agreement with other previously published studies.[18,21, 22] It seems that the normal Iranian diet has an adequate content of selenium for both genders.[18]

Zn and Cu are important trace elements which regulate the physiological function of various organs and are associated with the production of pathological changes in the organs.[23] Zinc is a trace element which influences the growth and affects the development and integrity of the immune system.[24, 25] Many studies have shown that Zn deficiency can cause T-cell dysfunction and impair cellular immune functions.[26] In this study, serum Zn levels were decreased substantially in acute leukemia patients when compared with the controls. There have been similar reports from other studies in which serum Zn levels were analyzed in patients with leukemia.[27, 28] We found no differences in Zn levels between subgroups of acute leukemia.

In the present study, there were no significant differences in Cu levels between acute leukemia patients and control subjects. These results are different from previously published studies.[11, 12] Cu is involved in biologic function which is related to its redox properties as a transition metal. Redox cycling between Cu^{2+} and Cu^{+} could ultimately produce highly toxic hydroxyl radicals.[29]

It is demonstrated that elevated Cu/Zn ratios in patient group could be a prognostic factor to determine the severity of disease.[6] In this study, the Cu/Zn ratio was higher in patients with acute leukemia than the controls. These findings are in agreement with other studies.[11] The Cu/Zn ratio might be related to the enhanced leukemic burden in several leukemia patients.

There was a positive correlation between the levels of serum Zn and Cu. Caglayan et al. reported a negative correlation between Zn and Cu in rheumatoid arthritis patients[30], whereas in another study, a positive correlation was reported.[31]

Rapid redistribution of Se, Zn, and Cu in the

body can occur in response to inflammatory processes by decrease in the levels of albumin, macroglobulin and other binding- proteins.[8] Albumin and hemoglobin are Se containing proteins [32]. In our study, there was no correlation between Se levels and albumin that could be as a result of small sample size. These findings are in agreement with studies done in certain cancers [33], but unlike other observations in patients with cancer and other diseases.[34, 35]
As expressed before, selenium is present in hemoglobin and is concentrated in RBC more than in the plasma.[7] We found a correlation between hemoglobin and serum Se in patients with acute leukemia. Further investigation is needed if Se supplementation could be helpful for the management of anemia in patients.
Because of many stresses during treatment such as hospitalization, complications of chemotherapy and bone marrow transplantation (BMT), risk of trace elements deficiency will increase as observed in previous study of author on patients who underwent BMT.[15]
In conclusion, this study showed a reduction in levels of Se and Zn in acute leukemia patients and because the complications of their deficiency may worsen the patient`s situation, we suggest further study to assess the effect of these trace elements on clinical outcome of disease. A difference in Se levels between different subgroups of AML patients was also found in this investigation and warrants further investigation in larger sample size studies. However, available data do not justify trace element supplementation programs in the whole population.

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