Short communication

Trace elements in blood of leukemia patients

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Abstract: Trace elements are minerals present in living tissues in small amounts. Some of them are known to be nutritionally essential, others may be essential, and the remainder are considered to be nonessential. Trace elements such as zinc, copper, selenium, iron, chromium and, others are essential elements for growth and body health. They form an integral part of many enzymes and bioactive centers that are responsible for biochemical reactions such as metabolism, protein and, DNA synthesis. They are said to contribute to the development of cancer based on epidemiologic evidence. The blood level of trace elements in the human body is affected by cancer and drug treatment. The low trace elements level is a sign of leukemia patients, where cancer cells consume body nutrients and essential elements for growth. The Libyan patients were divided into four age groups and were either newly or old diagnosed with leukemia, including patients with acute leukocyte leukemia, acute myeloid leukemia, chronic myelogenous leukemia, and aplastic anemia. The samples were tested for direct aspiration onto inductively coupled plasma spectrometry at specific wave lengths. The findings showed the blood level of trace elements in leukemia patients is lower than in the healthy individuals. A negative correlation between copper and zinc levels in leukemia patients was found. The copper level increases in leukemia patients as they get older.

Introduction

Inorganic elements are one of the important materials for the human body, and are essential components of enzymes, and proteins, and play regulatory roles in a large number of biological reactions. These elements are divided into abundant elements (major elements) and trace elements. Abundant elements are involved in the formation of covalent bonds and are important constituents of tissues. Major elements account for 96.0% of the total body weight and their deficiency can lead to nutritional disorders. The essential trace elements of the human body include zinc (Zn), copper (Cu), selenium (Se), iron (Fe), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), molybdenum (Mo) and nickel (Ni). Although these elements account for 0.02% of the total body weight, but they play a significant role in the live system such as active centers of the enzymes, and bioactive substances and, are essential for the growth of organisms due to their electrochemical and catalytic effects. Zn is an essential element, involved in numerous cellular metabolisms [1]. It catalyzes the

activity of several enzymes [2, 3] such as carbonic anhydrase and carboxypeptidase enzymes. Zn regulates CO_2 and protein digestion, respectively [4, 5]. It is involved in DNA synthesis, cell division, protein synthesis, immune function and, wound healing [3, 6]. Fe catalyzes many redox reactions and forms an integral part of different classes of proteins such as Fe-heme proteins, Fe-sulfur enzymes, Fe storage-transport proteins and, Fe-activated enzymes [7]. Cu is an essential element for maintaining the strength of skin, epithelial and, connective tissues and blood vessels throughout the body. It acts as an antioxidant to neutralize the free radicals that can cause tissue damage [8 - 10]. Cu acts as the reductant in enzymes like superoxide dismutase, cytochrome oxidase, lysiloxidase, dopamine hydroxylase, and several other oxidases. Mg is mostly found in bones, muscles and non-muscular soft tissue [11]. It is a cofactor of more than 300 enzymatic reactions such as catalytic enzymes in the glycolysis pathway [12, 13] and critically stabilizes enzymes, including many ATP-generating reactions [14]. An interference with

Mg metabolism may influence ATP-dependent processes [15]. Mg is considered a natural calcium antagonist [16] as it antagonizes the calcium-dependent release of acetylcholine at motor endplates [17]. Se is known as a cofactor of seleno-proteins such as glutathione peroxidases (GSH-Px) a family of antioxidant enzymes [18, 19] and it is an important component of thioredoxin reductase [20]. Se is present in red blood cells and various blood proteins like hemoglobin and albumin [21, 22]. Studies using experimental animals have shown that Se has anticancer activity, it reduces tumor recurrence, inhibits cell growth and angiogenesis, stimulates apoptosis, protects against oxidative damage and increases the immune function [23]. Cr is present in two oxidation states +3 and +6. Cr^{+3} is found in some enzymes such as chromodulinm, one of the most important enzymes for amplifying the insulin signaling effect while Cr^{+6} is potentially toxic [24]. A deficiency of trace elements in the body is accompanied by a reduction in the activity of the concerned enzymes (metalloenzyme) or other bioactive centers. Since each trace element is related to so many enzymes, deficiency of a single trace element is often not associated with any specific clinical manifestations, but rather manifests as a combination of various symptoms, which is often difficult for clinicians to identify deficiencies of some particular trace elements. Generally, the level of trace elements in the body is implicated in some diseases including cancer, diabetes, hypertension and, heart diseases [25]. Treatment of cancer with chemotherapy and radiation therapy causes many side effects and damages the nutritional status of the body [26]. These side effects of cancer therapy depend on the type of cancer, location, drugs, dose and the general health of the patient. The level of trace elements is sometimes affected by cancer chemotherapy because some drugs target some enzymes or active centers by acting or inhibiting these active sites in the human body. Monitoring of trace elements level during the treatment of cancer and elements intake will enhance the treatment of cancer. Given the important roles played by selected metals in various biological processes, this study is formulated to determine the levels of trace metals in blood of leukemia patients in comparison with healthy donors and to trace the relations among the measured metals in terms of correlation coefficient matrix and cluster analysis [27].

Materials and methods

Forty-one Libyan participants were included in this study and were divided into two groups: The control group consisted of 17 healthy subjects of both genders four males (23.5%) and 13 females (76.5%). The age of the control group was between 16 and 76 years. The patient group consisted of 24 subjects seven males (29.2%) and 17 females (70.8%), their age was between 16 and 85 years.

In **Table 1**, all the participants were divided into four age groups. The patient group was either newly or old diagnosed with leukemia, including patients with acute leukocyte leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), and aplastic anemia. The ethic approval to carry this study was obtained from the committee of university of Tripoli (e.c. 75-2022) as well as the participants were informed personally about the study and a written consent from each participant was obtained before starting sample collection. A written self-designed questionnaire was given to each participant, the questionnaire included several questions about age, gender and, chronic disease, additional questions were added to the patient group questionnaire including how long has he/she been diagnosed with blood cancer. Illness onset, type of leukemia and if they started a pharmacological treatment (chemotherapy, radiotherapy, or both). The patient group in the study was under chemotherapy.

Table 1: Age distribution of the controls and patients

Age (years)	Group	Mean age ± S.D.	Gender
16 - 34	Patient	16.0 ± 8.0	Male
		30.6 ± 4.0	Female
	Control	23.0 ± 4.0	Male
		23.8 ± 5.0	Female
35 - 50	Patient	45.2 ± 4.0	Male
		46.8 ± 2.9	Female
	Control	44.0 ± 8.0	Male
		45 ± 6.0	Female
51 - 65	Patient	65.0 ± 4.0	Male
		61.5 ± 4.0	Female
	Control	57.0 ± 9.0	Male
		62.3 ± 5.0	Female
> 65	Patient	70.0 ± 6.0	Male
		78.3 ± 7.0	Female

Blood collection: The blood samples (5 ml) were drawn from the participants in sterile green blood tubes and were immediately frozen after collection and analyzed within 72 hours. The blood samples were collected between July and September, 2022 from people who lived in Tripoli, Libya. The patient group attending and treated for cancer was from Tripoli Medical Hospital and Tripoli Central Hospital. The chemicals were used as received from vendors such as nitric acid (65.0% HNO_3), perchloric acid (37.0% $HClO_4$), and deionized water.

Instrument and equipment: Inductively Coupled Plasma Spectrometry (ICP) Agilent 5110 icp-0ES, Agilent SPS4 Auto-sampler and hotplate were used in this study. All glass and plastic wares were soaked in 05.0% (v/v) analytical grade HNO_3 overnight, rinsed with deionized water and dried in the oven. The equipment was appropriately stored to avoid contamination and dust. The analysis of samples for trace elements was measured using ICP in Libyan Petroleum Institute, Tripoli, Libya.

Methods for analysis of blood trace elements blood (BTE): The procedure has previously been described by Tariq and others [27] and was followed for the analysis of BTE. In a clean dry borosilicate beaker, 1.0 ml of blood sample was added, equimolar quantities of HNO_3 and $HCLO_4$ were added and the

mixture was heated on a hotplate (70 °C) till a clear solution was obtained [28]. The clear solution was diluted in a volumetric flask with deionized water up to 50 ml. A blank was prepared in the same way as the sample without blood. The solutions of samples and blank were used for direct aspiration onto ICP at specific wave lengths (nm), for each trace element: Cr (267.716), Cu (327.395), Fe (238.204), Mg (279.553), Mn (257.610), Se (196.026) and Zn (213.857). The level of trace elements under study was measured in mg/L.

Statistical analysis: Descriptive statistic was used to calculate the mean ± S.D. of data. A comparative statistical analysis was done by using an independent sample *t*-test. All the data was treated by using the IBM SPSS Version 21 program for data analysis.

Results and discussion

Cancer cells grow and divide uncontrollably consuming body nutrients, and energy and losing structure and function because of the inability to adequately differentiate. The mean level of blood trace elements and the distribution of trace elements of leukemia patients and their age and gender-matched controls are presented in **Table 2**.

Table 2: Blood trace elements for leukemia patients and control age groups

Groups		Blood trace elements in mg/L						
		Cr	**Cu**	**Fe**	**Mg**	**Mn**	**Se**	**Zn**
Patient	**16 - 34**	< 0.0002	1.313 ↑	0.209 ± 0.06 ↓	1.092 ± 0.17 ↓	< 0.0003	< 0.04	0.20 ↓
Control	**16 - 34**	< 0.0002	0.857	0.304 ± 0.06	1.25 ± 0.14	< 0.0003	< 0.04	0.23
P value		**N.A.**	**0.149**	**0.048**	**0.039**	**N.A.**	**N.A.**	**0.098**
Patient	**35 - 50**	< 0.0002	1.337 ↓	0.284 ± 0.02 ↓	0.941 ± 0.17 ↓	< 0.0003	< 0.04	0.86 ↑
Control	**35 - 50**	< 0.0002	1.608	0.3 ± 0.02	1.24 ± 0.36	< 0.0003	< 0.04	0.40
P value		**N.A.**	**0.566**	**0.036**	**0.008**	**N.A.**	**N.A.**	**0.576**
Patient	**53 - 65**	< 0.0002	1.227 ↓	0.253 ± 0.06 ↓	1.100 ± 0.4 ↓	< 0.0003	< 0.04	1.21 ↑
Control	**53 - 65**	< 0.0002	1.306	0.312 ± 0.05	1.21 ± 0.2	< 0.0003	< 0.04	0.34
P value		**N.A.**	**0.587**	**0.042**	**0.596**	**N.A.**	**N.A.**	**0.481**
Patient	**> 65**	< 0.0002	1.375 ↑	0.198 ± 0.1 ↓	1.01 ± 0.14 ↓	< 0.0003	< 0.04	0.21 ↓
Control	**> 65**	< 0.0002	1.306	0.247 ± 0.07	1.21 ± 0.2	< 0.0003	< 0.04	0.34
P value		**N.A.**	**0.524**	**0.579**	**0.790**	**N.A.**	**N.A.**	**0.460**
Normal range (ppm)		**0.00014-0.185**	**0.54-1.721**	**0.65-1.75**	**1.7-2.2**	**0.0004-0.015**	**0.042-0.19**	**0.05-0.15**
Significant at $P < 0.01$								
Significant at $P < 0.05$								

Generally, the mean blood trace elements levels of Fe, Se, Mg, and Cr values were significantly lower in the Libyan leukemia patients compared to healthy individuals. These findings support the data that have previously been reported [27, 29]. The mean levels for the patient group of Mg and Fe (0.244 mg/L, 1.026 mg/L, respectively) were found lower than the healthy group and the mean level of Cu was higher than the control group (1.29 mg/L) **(Table 3**, **Figure 1)**. The order for mean levels of BTE of leukemia

patients was found to be Cu > Mg > Zn > Fe > Se > Mn > Cr, in contrast to the healthy controls, where the observed order as Mg > Cu > Zn > Fe > Se > Mn > Cr. A significant difference in the mean level of Mg was observed in the young group (16 - 34 years) and middle (35 - 50 years) age groups ($P < 0.05$ and $P < 0.01$, respectively). These results explain the Mg level in leukemia patients significantly decreases with an increases in age. About 50.0% of the total body Mg is found in bone while the other half is

found inside cells of body tissues and organs and only 01.0% of Mg is found in the blood [30]. The clear reduction of Mg levels in female leukemia patients supports that Mg deficiency alters Ca metabolism and hormones that regulate Ca [31]. Also, Mg deficiency is considered a risk factor for postmenopausal osteoporosis. Fe for all age groups showed a significant difference with low mean concentration in the cancer patients than in healthy subjects (**Table 2**). The difference due to the samples that were been taken from patients when they are in flow up visiting the hospital for blood transfusion [32]. A high copper blood level was observed in leukemia patients compared to the healthy individuals in this study. The blood concentrations of Zn and Cu vary with inverse relation, when the Zn

level is elevated more than the control, Cu declined less than the control as shown in **Table 2**. Also, there is a negative correlation between Zn and Cu. This negative correlation between Zn and Cu has also been reported previously in rheumatoid arthritis patients [33, 34]. From **Table 2**, the Cu level in the blood increases as the leukemia patient gets older. All the participants (patients and controls) showed a lower concentration of Se, Mg and Cr than the normal range. The generally low level of trace elements in cancer patients is due to the impact of cancer growth and chemotherapeutic agents. Chemotherapy treatment destroys and damages body resistance (immune system) and digestive system (metabolic reactions) situation of living.

Table 3: Control and patient mean trace elements in all the participants

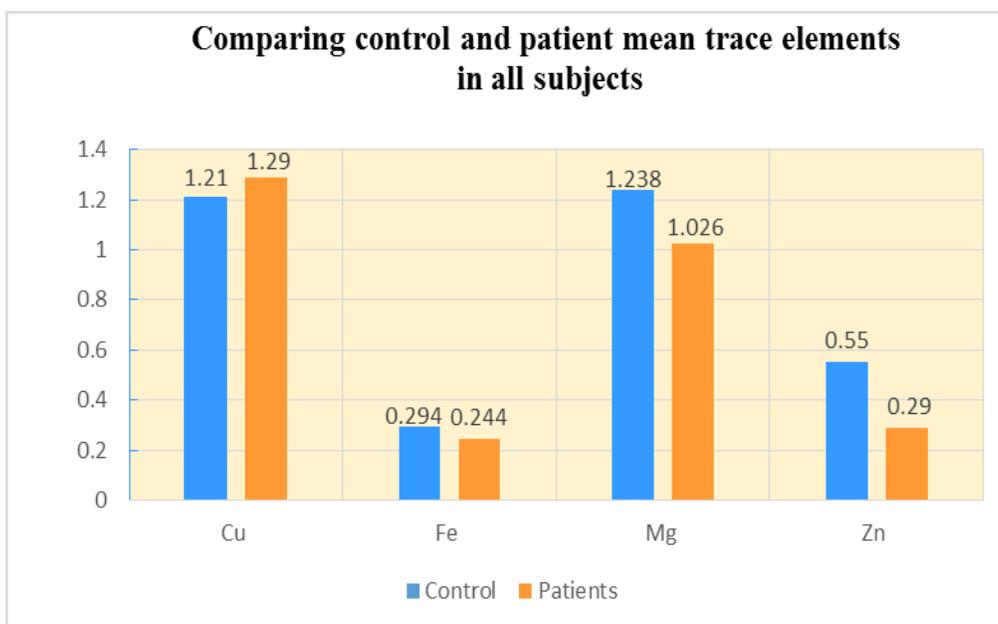| Groups	Cu	Fe	Mg	Zn
Control	1.21	0.294	1.238	0.55
Patient	1.29	0.244	1.026	0.29

Figure 1: Mean blood level of trace elements in the participants

Conclusion: This study confirms that the mean blood level of trace elements in Libyan leukemia patients is lower than in healthy controls. This low level may due to the consumption of trace elements by cancer

growth or chemotherapeutic treatment. A negative correlation between copper and zinc blood levels in leukemia patients is observed. The copper blood level increases in leukemia patients as they get older.

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