Evaluation of Copper, Zinc, Cu/Zn, and VEGF in Patients with AML in Iran

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Abstract

Background: Copper and zinc are the elements with numerous physiological activities. Copper (Cu) has an important role in angiogenesis and acts by increasing Vascular Endothelial Growth Factor (VEGF). Serum levels of copper will be increased in cancer incidence, progression and recurrence. The aim of this study was to measure blood levels of copper, zinc, and the ratio of Cu /Zn, as well as VEGF levels before and after treatment of acute myeloid leukemia.

Methods: Thirty patients who were recently diagnosed with Acute Myeloblastic Leukemia (AML) in Shahid Ghazi Tabatabai oncology hospital enrolled in this clinical trial. On the first day, blood samples were taken for copper, zinc, and VEGF assay and flowcytometry. Treatment protocol was (7×3) regimen. Blood samples were collected for evaluation of copper, zinc, and VEGF. They were sent to Biochemistry Laboratory in medicine faculty for analysis.

Results: Amongst 30 AML patients, 14 (46.7%) were female and 16 (53.3%) were male. Patients of various ages ranged from 16 to 53 years, with a median age of 9.1±9.35 years. The mean serum level of copper, zinc, and mean Cu/Zn ratio before and after treatment showed significant difference ($p<0.05$) There was also significant difference between the mean VEGF level before and after treatment ($p<0.05$).

Conclusion: This study reveals that there is no significant relationship between copper, zinc serum levels, their ratio, and VEGF in AML patients. We hypothesize that increased serum copper is associated with increase of VEGF levels which can indicate the impact of copper in malignancies including AML.

Key words: Copper; Zinc; Vascular Endothelial Growth Factor A

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Introduction

The cells of different organs need oxygen and nutrients to survive. Judar Falk man expressed that expansion of tumor tissue needs vascular growth [1]. Angiogenesis is divided into two groups: Physiologic and Pathologic [2-5]. In normal conditions there is a balance between angiogenesis and anti angiogenesis [1, 4, 6]. Angiogenesis factors act firstly by production of angiogenesis activation growth factors, secondly by activation of metalloproteinase, thirdly by immigration of endothelial cells, and finally by Inhibitions of endothelial cells apoptosis [1, 3].Various mechanisms induce angiogenesis like stress, inflammation, gene mutation, and sepsis [6-7].

The most important factor is metabolic stress especially hypoxia. The most important well known growth factor in angiogenesis is VEGF [7].

Copper is the one of the elements which has various physiologic functions in the body [8]. Copper is involved in enzyme functions, Cu/Zn super oxide dismutase, ferroxidase, cytochrome oxidase-C, tyrosinase, dopamine hydroxylase, and lysine oxidase functions. These enzymes are involved in cellular respiratory chain, defense against free radicals, melanin synthesis, endothelial tissue growth, Iron metabolism, and gene expression [1, 2, 6-8].

Zinc is the one of the elements involved in function of about 200 metalloenzymes [8, 9]. Zinc releases cytochrome C from mitochondria so that some

enzymes named caspases will be activated which finally result in apoptosis [10, 11]. Zinc also causes reduction of tumor cells and tumor size [12].Copper in physiologic range provides cellular health and in higher than physiologic concentrations causes angiogenesis [3]. Copper, as an angiogenic factor, causes higher incidence of cancers while zinc, as an apoptotic agent, reduces incidence of malignant diseases. Copper/zinc ratio is very important because there is a high competition between these two elements to enter the cells [12- 13].

Zarghami has reported that changes in copper and zinc levels have an important biologic role in developing breast cancer [14]. In another study, Zarghami indicated that changes in copper and zinc serum levels may have a biologic role to initiate and development of tumoral tissues [15]. Copper concentrations in serum increase incidence of malignant diseases and also have a role in development and recurrence of Hodgkin lymphoma, sarcoma, and leukemia and also lung tumor as well as liver and breast tumors [13]. One of the malignancies which is associated with increased angiogenesis is acute myeloid leukemia [16, 17].The aim of this study was to evaluate serum levels of copper, zinc, and copper/zinc ratio as well as VEGF levels before and after treatment of AML patients. If copper level in serum does not decrease after treatment, in future studies we would decrease the angiogenesis by adding drugs reducing copper levels to improve survival.

Materials and Methods

This was a randomized clinical trial in Hematology-Oncology Research Center in Tabriz, Iran conducted between 2006 and 2008 after approval from the scientific review committee and institutional review board. All patients signed informed consent. Patients with AML in hematology ward were eligible for this study. All patients with AML (except AML M_3) took chemotherapy with combination of cytosine arabinoside (Ara-C) and anthracyclin. Patients received 100 mg/m² Ara-C daily for 7 days and also received either 45 mg/m² daunorubicin (DNR) or 10 mg/m² idarubicin per day in first 3 days of treatment (7+3 regimen). Before chemotherapy and after complete remission, blood samples were taken from all patients for measuring copper, zinc, and VEGF levels. They were frozen in -80ᶜ. We measured VEGF with Enzyme-linked immunosorbent assay (ELISA) by IBL (Hamburg, Germany) kits; copper and zinc were measured by ELISA method by randox (Randox LAB, UK) kits. SPSS 13 software, Pearson correlation and Kaplan-Meier tests were used for statistical analysis and P<0.05 was considered significant.

Results

Thirty patients with AML were studied in this research.
14 patients were female (46.7%) and 16 patients were male (53.3%). Patients were in the age range of 16 -53 years (mean age of 35.9± 10.9 years). Changes in copper, zinc, and VEGF levels before and after treatment are shown in (Table 1).

Kolmogor-smirnor test was used for evaluating normality of data and revealed that copper, zinc, and VEGF levels had normal distribution before and after treatment. In statistical analysis, there was no significant relation between age and gender regarding copper, zinc and VEGF levels. There were significant differences in copper levels (P=0.029), zinc levels (P=0.0001), VEGF levels (P=0.009), and Cu/Zn ratio (P=0.002) before and after treatment. There was no significant relation between copper, zinc, and VEGF levels before and after treatment, but a significant relation was detected between Cu/Zn ratio before treatment (P=0.0001) and copper after treatment (P=0.009).

Discussion

Copper is an element with various physiologic functions [1, 9]. It has an important role in angiogenesis [7-8]. In a study by Camphausen, CuSo4 was inserted into the anterior chamber of rat eye which caused angiogenesis in the eye [5]. Previously copper was known as a chemotactic factor but Brewer reported that copper induces fibronectin release from endothelial cells in a cell culture and this substance accumulates on the surface of endothelial cells and causes adhesion of vessels endothelial cells [2]. In a study by Harris, increased levels of copper were reported in breast adenocarcinoma cells which confirm the effect of copper in angiogenesis and metastatic tissues [3].

Table 1. Changes in copper, zinc and VEGF levels before and after treatment

	The mean of the changes before treatment	The mean of the changes after treatment
Cu(μg/dl) Zn	65.76±23	155.09±66.72
(μg/dl) VEGF	77.47±29.45	120.81±57.48
(pg/dl)	193.78±202.9	140.61±115.20
Cu/Zn	0.89±0.48	1.42±0.37

Cu: Copper, Zn: Zinc, VEGF: Vessel Epithelial Growth Factor

In our study, significant differences were observed in mean copper levels before and after treatment. There was also a significant difference in zinc levels before and after treatment ($P<0.05$). The differences between Cu/Zn ratio and copper level were significant before ($P=0.0001$) and after treatment ($P=0.009$), confirming the competition between these two elements for entering the cells. No relation was found between Disease Free Survival (DFS), Overall Survival (OS), copper, zinc, and VEGF before and after treatment.

Copper induces blood vessels growth and angiogenesis by increasing VEGF [1-3]. Leukemia is associated with increase of new blood vessels [16-17]. In our study a significant difference was observed in VEGF levels before and after treatment ($P<0.05$) which confirmed the previous studies.

In our study there was no significant relation between copper, zinc, Cu/Zn, and VEGF and AML. This was unlike previous studies in which a direct relation between copper and VEGF level was reported; and this may be due to a small number of patients. In the literature review, no similar study was found to evaluate the copper, zinc, and Cu/Zn ratio before and after treatment in AML; and our study seems to be the first in this regards. It is however suggested to design a study with more patients and adding copper decreasing agents to study the effect of decreasing copper levels on malignant disorders.

Acknowledgement

Conflict of Interest

This study has been sponsored by Hematology and Oncology Research Center of Tabriz University of Medical Sciences.

Authors' Contribution

ZS designed the study, collected the data and wrote the paper. MN contributed to the laboratory test.BH and RD contributed to the data entry. IA, JV, JE, AN, AE, and SHC contributed to the patients management. HB contributed to the analyzed and interpreted the data. All authors read and approved the final revision.

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