ABSTRACT

Background: Zinc acts as growth protector for neoplastic cells and its deficiency was contributed to carcinogenesis. However, the determinations of serum zinc in acute lymphocytic leukemia (ALL) prediction and prognosis requires more investigations. Objective: To evaluate and compare serum zinc in ALL patients and healthy controls and to correlate the serum zinc levels with hematological prognostic markers. Materials and methods: the study was conducted in Khartoum state-Sudan during the period from December 2013 to September 2014, it involved a case group of ALL patients (N=100) matched for age and gender with a control group (N=100). Serum copper and zinc levels and full blood count were investigated. Results: The ALL patients showed lower levels of Zn \(0.73 \pm 0.18\) mg/dl compared to controls \(1.01 \pm 0.25\) mg/dl \([P = 0.003]\). The serum Zn levels were inversely correlated with total white cell \((-0.804, P < 0.0001)\) and blast counts \((-0.935, P < 0.0001)\). Conclusion: These findings are associated with lower serum zinc levels and higher serum copper levels. The determination of serum zinc and copper could be used as ALL prognostic markers.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignancy of the lymphocyte progenitor cells or the lymphoblasts which accounts about 75% of childhood leukemias and 20% of leukemias in adults [1]. Till now, the exact cause of ALL remains unknown, but it is likely to involve a complex interaction between genetic susceptibility and environmental exposure[2]. The pivotal role of trace elements in the process of normal proliferation and differentiation of various tissues in animals and humans has been reported[3]. As they may affect tumor cell proliferation, their levels are considered to be of significant importance in carcinogenesis[4]. Some of these elements exert their preventive role against malignant proliferation by participating protection against oxidative stress exerted by free radicals in the cells that contribute to cancer development[5].

Zinc (Zn), an essential trace element, a component of more than 200 enzymes, plays an important role in the maintenance of several tissue functions[6]. Zn is an essential mineral that is integral to many transcription factors that regulate key cellular functions such as the response to oxidative stress, DNA replication, DNA damage repair, cell cycle progression, and apoptosis. In particular, several proteins involved in DNA damage signaling and repair, replicative enzymes such as DNA and RNA polymerases and transcription factors such as tumour protein p53[7]. Trace amount of Zn is required in the diet, which is around 15 mg Zn/day[8], with a total amount in the adult body being in average of 1.4-2.3 g [9]. The estimation of circulating levels of Zn either in plasma or serum has been the most widely used approach for the assessment of zinc nutrition[10]. It has been reported that changes in zinc serum levels may play an important biologic in the initiation and development of tumor mass[11].

MATERIAL & METHOD

Study design: The study was analytical observation study
Ethics approval: A written consent was obtained from the parents/ caregivers of the subjects and controls after explaining to them, in detail, the objectives of the study as well as the method of specimen collection.
Sample size: One hundred patients with ALL as case group and 100 apparently healthy individuals as control group were included in this study. The controls were age- and gender-matched for cases. The patients were attendants of clinics and hospital in Khartoum state, Sudan from December 2013 to September 2014.

Case group: The diagnosis was confirmed by examination of both peripheral blood and bone marrow examination. All the patients were enrolled in the study before receiving the first course of chemotherapy. The control group was used only for comparing serum zinc.

Inclusion criteria: Both male and female Sudanese patients confirmed with ALL in Khartoum state at presentation at any age and before starting chemotherapy.

Exclusion criteria: Recent blood transfusion history, taking any medication with mineral supplement or drugs that affect iron metabolism or starting chemotherapy.

Blood sample collection: Five ml of fasting blood sample was collected from the antecubital vein of each of the case and control group. Three ml were poured into EDTA anticoagulated tube the rest of the sample was poured into plain tube. Samples with signs of hemolysis were discarded. The blood was then centrifuged for 15 minutes at 3000 rpm to extract the serum. The serum was aliquoted into deionised polyethylenetubes and stored at -80°C.

Full blood count (CBC): Full blood count was performed from EDTA samples within half hour after collection using automated blood cell counter to obtain total white cell count. A microscopic examination of peripheral blood film was done to get the blast count.

Biochemical Analysis: Serum concentrations of Zn in both patients and controls were determined by using atomic absorption spectrophotometry.

Statistical Analysis: One-way ANOVA was used for the comparison of mean values of the groups. Then, Student-t test was used to determine the difference between groups. Pearson correlation analysis was used to study the relationship between serum Zn with total white cell count and blast count. A “p” value <0.05 was considered statistically significant. Statistical analyses were carried out using the SPSS® statistical software package (SPSS for Windows version 21.0 SPSS Inc., Chicago, Illinois, USA). All results are expressed as mean and standard deviation (mean ± SD).

RESULTS

One hundred patients with ALL (54 male, 46 females; mean age 22.6 ± 18.8 years) and 100 healthy subjects (55 male, 45 female; mean age 19.9 ± 19.7 years) comprised the study group, table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=100)</th>
<th>Controls (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54</td>
<td>46</td>
<td>55</td>
</tr>
<tr>
<td>LDH</td>
<td>0.73 ± 0.18</td>
<td>1.01 ± 0.25</td>
<td>0.003</td>
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Figure 1. levels of serum Zn in ALL patients and controls

Pearson correlation analysis among ALL, showed a highly significant an inverse correlation between serum Zn levels and white blood cell counts (r = 0.46, p < 0.04) as well as with the number of blast cells (r = 0.8, p< 0.001), Table 2.

Table 2. Correlation of serum Zn with WBC and blast cell in ALL patients

<table>
<thead>
<tr>
<th></th>
<th>White cell count/ µl</th>
<th>Blast cell count/ µl</th>
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<tbody>
<tr>
<td>Mean ± SD</td>
<td>176.3± 45.8</td>
<td>122± 39.9</td>
</tr>
<tr>
<td>Pearson correlation (r)</td>
<td>-0.804</td>
<td>-0.935</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
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</tbody>
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DISCUSSION

The present study was designed to evaluate serum Zn in ALL patients. Also correlations have been made between the serum levels of Zn and total white cell count and blast count within the case group.

In addition to its major function and metabolic effect described above, the accumulation of high zinc levels can have other consequences. One such effect is the inhibition of cell growth, which results from its induction of mitochondrial apoptosis[12,13].

The maintenance of discrete subcellular pools of zinc is critical for the functional and structural integrity of cells. Among the important biological processes influenced by Zn is apoptosis[14].
It has also been identified as a major mechanism contributing to cell death in response to toxins and in disease, offering hope that novel therapies that target apoptotic pathways may be developed. Zinc becomes cytotoxic if its extracellular concentration exceeds the capacity of the Zn homeostatic system. Elevated extracellular Zn concentrations lead to the breakdown of the Zn transporting system of the plasma membrane. The resulting enhanced intracellular Zn concentration activates the apoptosis\textsuperscript{[19]}. Zn releases cytochrome C from mitochondria so that some enzymes named caspases will be activated which finally result in apoptosis\textsuperscript{[26]}. Zn also causes reduction of tumor cells and tumor size\textsuperscript{[17]}. Esophageal Squamous Cell Carcinoma with TP53 mutant tumor had lower zinc levels than those with no mutation\textsuperscript{[18]}. Our results indicate that the serum Zn in ALL patients is lower than in control subjects. This is in agreement with previous data\textsuperscript{[19,20]}. Our results indicating that serum Zn showed an inverse association with some hematological markers of ALL: total white cell count and blast count, which are used in index to predict the response to chemotherapy. The accumulation of high intracellular levels of zinc by tumor cells induces mitochondrial apoptogenesis\textsuperscript{[12]}. Consequently the availability of Zn in blast cells could induce its apoptosis and thus decreasing its count which is parallel to mass in solid tumor.

**CONCLUSION**

Zinc deficiency contributes to carcinogenic factors in ALL, thus the correction of reduced Zn could prevent or at least decrease the severity onset of the disease. The inverse association between Zn and total white cell and blast count could emphasize the role of incorporation Zn in treatment protocol.

**Acknowledgment**

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**REFERENCES**


*Omar et al.*