(TABASSUM-C)

## Research Article

# Assessment of Some Selected Trace Metals in Chronic Myeloid Leukemia Patients in a Tertiary Health Facility in South West Nigeria

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**Abstract:** Some selected trace metals; Iron (Fe), Copper (Cu), Manganese (Mn), Chromium (Cr), Selenium (Se), Calcium (Ca), Zinc (Zn) and Magnesium (Mg) in Chronic Myeloid Leukemia (CML) patients were assessed. Seventy five (75) leukemic patients and 50 apparently healthy control subjects were evaluated in this study. Hematological parameters were estimated by automation SYSMEX KX-21N and the trace elements analysed using Atomic Absorption Spectrophotometry. There were statistical significant differences between serum Fe, Cu, Mn, Se, Ca, Zn and Mg of leukemic patients and that of their respective control subjects ($p < 0.05$) while there was no significant difference between serum chromium of leukemic patients and the control subjects ($p > 0.05$). Variation also existed between the patients and control values in the hematological parameters such as: PCV, MCV, MCHC, WBC, platelet etc. The study reveals that serum trace elements are useful indices of the extent of the disease in leukemia patients, their levels are independent of a non specific acute-phase reaction and restoration of serum trace elements are useful in assessing response to treatment in leukemia patients. Monitoring of the respective trace elements in the CML patients could therefore be an essential part of managing the patients.

**Keywords:** Chronic myeloid leukemia, hematological parameters, trace metals

## INTRODUCTION

Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal, uncontrolled proliferation and process of malignant transformation of one of the hematopoietic cells, usually white blood cells (leukocytes). It is part of the broad group of diseases called hematological neoplasm. The abnormal proliferation of hematopoietic cells causes progressive infiltration of bone marrow, although in certain forms the lymphatic tissues are particularly affected. The process of differentiation in Leukemia cells is often abnormal, and this commonly results in an immature morphological appearance. The rate of progression varies considerably in different types of leukemia, but death is the usual outcome in untreated disease as a result of compromised production of mature blood cells (Ramnik, 2003).

Leukemia was also described as a group of cancer that begins in blood cells, characterized by an uncontrolled, abnormal, irreversible, progress of malignant transformation and widespread proliferation of the leucocytes of the body, which infiltrate the bone marrow, a haemopoietic tissue, and other organs, and usually in the blood in increased numbers. Like other cancers, leukemia is a disease of unknown aetiology; however, research has shown that people with certain risk factors are more pathologically divided into several groups. The first division is between its acute and chronic forms. It is classified by the type of blood cell affected (Hoffbrand *et al.*, 2006).

Chronic myeloid leukemia accounts for 20% of all leukemia affecting adults. It typically affects middle age, uncommon in young individuals. The main characteristics of this mutation are the formation of a fusion gene (BCR/ABL) with increased tyrosine kinase activity from which a transforming activity for myelopoiesis derives. The resulting BCR/ABL fusion gene encodes a chimeric protein with strong tyrosine kinase activity expression if

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this protein leads to the development of chronic myeloid leukemia phenotype (Emmanuel, 2009). The presence of BCR/ABL rearrangement is the hallmark of chronic myeloid leukemia. It is also considered diagnostic when present in a patient with clinical manifestation of chronic myeloid leukemia (Hirase, 2009).

Clinically, chronic myeloid leukemia is characterized by an initial chronic phase followed by inevitable progression to an accelerated phase and then to a terrible blastic phase. Chronic myeloid leukemia usually present in a chronic phase of variable duration, after which a fatal condition similar to acute leukemia (Blastic crisis) develops (Kantarjian and Talpaz, 2004).

Trace elements are essential nutrient for human being. They are crucial for the functioning of several enzyme systems. They are involved in gene expression, RNA and DNA metabolism, cellular immune functions, and also play a role in cellular protection against free radical injury. They also terminate the chain reaction formed by free radical which leads to cellular damage. Thus they are of fundamental importance in living organisms. Trace elements are so called because they constitute less than 0.01% of the weight of the human body. Importance of human nutritional iron and trace metals deficiencies have been recognized for many years. Recently, it has become apparent that human beings can suffer adverse effects from nutritional deficiencies of essential trace elements including zinc, copper (Shaw, 1980).

Zinc is a constituent of DNA and RNA polymerase; it is required as a catalytic, structural, and regulatory ion for enzymes, protein and transcription factors and thus a key trace element in many homeostatic mechanisms of the body, including immune responses (Prasad *et al.*, 1998). Zinc is a cofactor of all six classes of enzymes (Rink and Gadrie, 2000). Signs and symptoms of zinc deficiency includes depressed growth with stunting, increase incidence of infection, possibly related to alteration in immune functions, diarrhea, skin lesions (Carl *et al.*, 2008).

Excessive dietary intake of zinc has been linked with declining CD4 counts and reduced survival (Baum *et al.*, 2003). High dosages of zinc evoke negative effects on immune cells and cause alteration similar to those observed in zinc deficiency (Chaturvedi *et al.*, 2004).

Selenium is also essential for normal functioning of the immune system. Plant foods are major dietary source of selenium and also metals and sea food (Levander and Beck, 1997). Selenium also serves as a cofactor for glutathione peroxidase, an important enzyme in the antioxidant cascade and cellular protection, the low levels of selenium found in some cases of cancer may reflect long-term impairment of cell protection capacity (Bratako *et al.*, 1990). Selenium deficiency has been directly associated with the development of cancer, cardiovascular disease and hepatic lesions (Simonoff *et al.*, 1992).

Deficiency of selenium is accompanied by loss of immunocompetence and this is related to the reduction of selenoprotein in the liver, spleen, and lymphnodes. Both cell-mediated immunity and B-cell function are impaired (Carl et al., 2008). Decreases in Selenium, Zinc and a high Copper status have been demonstrated in leukemia patients (Zuo et al., 2006).

Copper is an essential trace metal in the body. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reaction and in scavenging free radical (Wang *et al.*, 1998). Almost all the copper in the body is present as a component of copper-carrying protein called ceruloplasmin. It acts as a catalyst in the formation of haemoglobin. Most common clinical signs of copper deficiency are anaemia that is unresponsive to iron therapy but corrected by supplementation which result from defective iron metabolism due to decreased ceruloplasmin activity (Rukgaver *et al.*, 2002). Copper is considered a pro-oxidant element and could increase the cellular damage by an interaction with reactive oxygen species (Rotilio *et al.*, 2000). Although normally bound to protein, copper may be released and become free to catalyze the formation of highly reactive hydroxyl radical that have capacity to initiate oxidative damage and interfere with important cellular events (Baum *et al.*, 2003). An increased Cu/Zn ratio could be an index of unfavorable prognosis of acute leukemia (Zuo *et al.*, 2006).

Calcium is a structural component of bones, teeth, and soft tissues and is essential in many of the body's metabolic processes. It accounts for 1 to 2% of adult body weight, 99% of which is stored in bones and teeth. On the cellular level, calcium is used to regulate the permeability and electrical properties of biological membranes (such as cell walls), which in turn control muscle and nerve functions, glandular secretions, and blood vessel dilation and contraction. Calcium is also essential for proper blood clotting (Berkow and Robert, 1997). Persistent alteration of calcium ions by magnetic field could influence the process of cancer promotion and affect the growth control in normal and malignant cells. An increase in the level of calcium has been demonstrated in disorders such as breast cancer, lymphoma and leukemia (Sakakibara and Morim, 1993).

Magnesium is the fourth most abundant mineral in the body and is essential to good health. Approximately 50% of total body magnesium is found in bone. The other half is found predominantly inside cells of body tissues and organs. Only 1% of magnesium is found in blood, but the body works very hard to keep blood levels of magnesium constant. Bone health is supported by many factors, most notably calcium and vitamin D. However, some evidence suggests that magnesium deficiency may be an additional risk factor for postmenopausal osteoporosis. This may be due to the fact that magnesium deficiency alters calcium metabolism and the hormones

that regulate calcium (Wester, 1987). Iron encourages the formation of cancer-causing free radicals. The body needs a certain amount of iron for healthy blood cells. But beyond this rather small amount, iron becomes a dangerous substance, acting as a catalyst for the formation of free radicals. Because of this, research studies have shown that higher amounts of iron in the blood could also mean higher cancer risk.

Numerous laboratory and clinical investigations over the past few decades have observed that one of the dangers of iron is its ability to favor neoplastic cell growth. The metal is carcinogenic due to its catalytic effect on the formation of hydroxyl radicals, suppression of the activity of host defence cells and promotion of cancer cell multiplication. In both animals and humans, primary neoplasms develop at body sites of excessive iron deposits. The invaded host attempts to withhold iron from the cancer cells via sequestration of the metal in newly formed ferritin. The host also endeavours to withdraw the metal from cancer cells via macrophage synthesis of nitric oxide. Quantitative evaluation of body iron and of iron-withholding proteins has prognostic value in cancer patients.

Manganese deficiency has been associated with cancer, rheumatic conditions, rickets, morning sickness, jaundice, and diabetes. Excessive ingestion of iron, combined with hypochlorhydria, can cause an imbalance in the Mn/Fe ratio. Manganese poisoning has been found among workers in the battery manufacturing industry. Symptoms of toxicity mimic those of Parkinson's disease (tremors, stiff muscles) and excessive manganese intake can cause hypertension in patients older than 40. Significant rises in manganese concentrations have been found in patients with severe hepatitis and post hepatic cirrhosis, in dialysis patients and in patients suffering heart attacks. Chromium occurs in any oxidation state from-2 to +6. Trivalent chromium (Cr+3) is the biologically active form, while hexavalent chromium (Cr+6) is potentially toxic to humans. Exercise and trauma also contribute to chromium loss, and it is interesting to note that in laboratory animals, extra chromium supplementation resulted in a life span increase of up to 33%.

Blood serum trace metals in malignant diseases have been the subject of a multitude investigation and their involvement has been well recognized in many cancerous conditions. This study is therefore aimed at assessing some selected trace metals in patients suffering from chronic myeloid leukemia in Nigeria.

## MATERIALS AND METHODS

**Subjects:** This study was carried out at Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. Seventy-five blood samples were selected for the study from routine samples submitted to the hematology laboratory for hematological malignancies investigation between March and June 2011. The samples which were diagnosed for chronic myeloid leukemia were included. Fifty apparently healthy individuals also served as controls.

Six milliliter (6 mL) of venous blood was withdrawn from each patient. Three milliliter (3 mL) was placed into an EDTA bottle for the estimation of hematological parameters and the rest into plain bottle from which serum was separated and stored in a plain bottle at $-20^0$C until analysis was done.

**Method:** Samples were stained for differential white cell count using the method of Dacie *et al. (*2006) while flow cytometry method was applied for the full blood count including total white cell count. Determination of trace metals was done using atomic absorption spectrophotometry as described by Bernhard (1985).

**Statistical analysis:** Descriptive and inferential statistics was employed using Statistical package for Social Sciences (SPSS Version 16.0) computer software to analyze raw data obtained. In evaluating the raw data, the following statistical tools was used, Mean (X), Standard deviation ($\pm$SD, $\pm$2SD), Analysis of variance (ANOVA) and test of significance (p-value). $p<0.05$ is taken as statistical significant difference between the groups.

## RESULTS

A total of 125 subjects consisting of 75 leukemic subjects 44 (58.6%) males and 31 (41.3%) females) and 50 healthy controls 30 (60%) males and 20 (40%) females were selected for the study. The subjects' age ranged between 15 and 85 years.

Apart from the clinical signs and symptoms employed for the diagnoses of leukemia, the mean and the standard deviation of the hematological parameters of both male and female leukemia patients (CML) with cell population and morphology were compared with controls for confirmation. There were higher levels of White blood cell (WBC) count, Red cell indices (MCV, MCH, MCHC), Neutrophil, Lymphocyte compared to their control counterpart in both male and Female patients. In all the hematological parameters estimated and considered in the diagnosis of leukemia condition, significant differences existed between the total white blood cells count, mean corpuscular volume and mean corpuscular hemoglobin of the leukemia patients of both sex and the controls $p<0.05$ (Table 1).

Mean serum selenium, calcium, copper, iron, manganese and zinc were significantly elevated in the patients compared to healthy individuals ($p<0.05$). However, serum magnesium concentration was significantly lower when compared with controls ($p<0.05$) (Table 2).

Table 1: Hematological parameters and indices in CML patients and control subjects

| Parameters | Leukemia patients female n = 31, X±SD | Male n = 44, X±SD | Control subjects | p-value |
|---|---|---|---|---|
| Age (years) | 62.48±15.89 | 58.23±15.73 | 25.80±5.59 | >0.05 |
| WBC count ($\times 10^{3/:L}$) | 45.24±26.89 | 31.29±20.05 | 4.918±1.35 | <0.05 |
| RBC count ($\times 10^{6/:L}$) | 3.82±0.76 | 3.82±0.76 | 4.7954±0.60 | >0.05 |
| Hemoglobin (g/dL) | 10.42±2 .05 | 10.74±2.27 | 10.74±2.27 | >0.05 |
| PCV (%) | 33.14±6.60 | 33.97±6.92 | 36.982±4.36 | >0.05 |
| MCV (FL) | 87.30±7.27 | 92.63±8.43 | 79.214±4.00 | <0.05 |
| MCH (pg) | 27.53±3.08 | 29.26±3.13 | 24.575±1.81 | <0.05 |
| MCHC (g/L) | 315.30±1.79 | 315.90±1.48 | 308.86±16.02 | >0.05 |
| Platelets ($\times 10^{3/:L}$) | 254.53±160.05 | 197.48±165 .06 | 208.18±87.90 | >0.05 |
| Neutrophil (%) | 58.18±22.35 | 48.50±25.28 | 47.096±7.27 | >0.05 |
| Lymphocyte (%) | 36.40±24.01 | 197.48±22.48 | 6.354±4.28 | >0.05 |
| Mixed (%) | 9.98±5.24 | 13.14±8.30 | 0.368±0.81 | >0.05 |

Table 2: The mean and standard deviation of estimated trace metals in leukemia (CML) patients and control subjects

| Parameters | Leukemia N = 75 X±S.D | Control N = 50 X±S.D | ANOVA | p-value |
|---|---|---|---|---|
| Fe (:g/dL) | 145.21±103.11 | 53.77±39.10 | 24.13 | <0.01 |
| Cu (:g/dL) | 1014.51±619.10 | 405.84±174.43 | 29.61 | <0.01 |
| Mn (:g/dL) | 46.05±19.85 | 18.45±11.20 | 79.6 | <0.01 |
| Cr (:g/dL) | 0.88±0.44 | 0.90±0.47 | 0.048 | <0.05 |
| Se (mg/dL) | 31.86±6.88 | 22.53±1.99 | 12.36 | <0.01 |
| Ca (mg/dL) | 9.32±0.23 | 5.41±0.14 | 135.99 | <0.01 |
| Zn (:g/dL) | 140.74±10.33 | 62.71±3.26 | 26.65 | <0.01 |
| Mg (mg/dL) | 7.07±0.37 | 13.55±1.45 | 13.66 | <0.01 |

## DISCUSSION AND CONCLUSION

Some selected trace metals (Zn, Se, Ca, Mg, Cu, Fe, Mn and Cr) and hematological parameters in CML patients were assessed in this study. Calcium concentration was significantly raised in the patients. This observation is similar to the report of study carried out by Lyle *et al*. (1991) which showed an increase in calcium level in the test patients which were significantly higher than the control. The increase in the level of calcium has been shown in other disorders such as breast cancer and lymphoma. The role of cellular calcium is to control cell signal and events such as cell movement and division.

The mean Selenium concentration of test patients was also increased when compared with the corresponding control. The reason for this increase is not very clear because it is observed that selenium deficiency leads to increased level of lipid peroxidation and therefore an increase in oxidative stress in human and animal tissue (Vanhanavikt and Ganthier, 1993). This suggests that an increase in selenium, may lead to decreasing level of lipid peroxidation since selenium containing antioxidant enzyme, glutathione peroxidase effectively reduces hydrogen peroxide to $H_2O$ and lipid alcohol respectively.

Perhaps, selenium was increased significantly to compensate for the damaging effect caused by the disease.

Higher levels of copper in comparison to controls were observed in this study. This is similar to what has been previously obtained in some studies. For instance, a value of 1.82 ug/mL was reported in a homogenous group of CLL patients (Pizzolo *et al*., 1978). A significantly higher level of copper in was also demonstrated leukemia patients (Zuo *et al*., 2006). The elevation of copper and caeruloplasmin in cancer patients has often been considered as part of a non specific acute phase reaction (Margerison and Mann, 1985) but this statement has been challenged by others (Linder *et al*., 1981). Evidence supports the value of serum copper as one of the indices of the extent of the disease, which appear to be independent of a nonspecific acute phase reaction.

The elevated serum zinc level observed in this study does not seem to agree with the normal inverse copper-zinc relationship in healthy persons and even in leukemic patients (Zuo *et al*., 2006; Beguin *et al*., 1987). The higher significant mean level for serum iron concentration in this study is consistent with earlier reports that confirm increased iron levels in persons with leukemia (Anntila *et al*., 1992). However, there is no significant difference found in the serum chromium levels between patients and controls in this study and this result is consistent with earlier reports for leukemia patients.

Conclusively, these results suggest that there could be alteration of some trace metals in chronic myeloid leukemia patients which may not necessarily be specific for the area under study. However, the trend of the trace metals may be used as biochemical markers in these patients. Further studies are warranted to determine the prognostic value of serum trace metals for the survival of CML patients.

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