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Original Article

Antioxidant Levels of Acute Leukaemia Patients in Nigeria

Olaniyi John A[1], Anifowose Agnes[3], Akinloye Oluyemi[3], Awosika Emmanuel O[1], Rahamon Sheu K[2] and Arinola Ganiyu O[2*]

[1]Department of Haematology, [2]Chemical Pathology and Immunology, University of Ibadan, Nigeria,
[3]Department of Chemical Pathology, LAUTECH, Ogbomosho, Nigeria

ABSTRACT

The incorporation of nutritional screening and comprehensive assessments of oxidative stress is increasingly recognised as imperative in the development of standards for quality care in oncology. This study evaluated the levels of nitric oxide (NO), some essential trace metals (Zn, Cu, Fe, and Se), superoxide dismutase (SOD) activity and malondialdehyde (MDA) in twenty five (25) patients with acute leukaemia and 25 apparently healthy controls. The mean levels of plasma Zinc (Zn), Iron (Fe) and Selenium (Se) were not significantly elevated ($p > 0.05$) in leukaemia patients compared with controls. Also, slightly lower level of plasma Cu was observed in leukaemia patients compared with the controls. However, nitric oxide was significantly increased ($p < 0.05$) in leukaemia patients compared with controls. The implication of the present finding is that intervention to increase antioxidant status in patients with Acute Lymphoblastic Leukaemia (ALL) should be considered.

Keywords: Antioxidant, Leukaemia, Lymphoproliferative, Malondialdehyde, Oxidative stress, Pathophysiology

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INTRODUCTION

Leukaemia, a haemopoietic cells' neoplasm, is bone marrow malignancies often characterised by abnormal increase in white blood cells. Leukaemia constitutes the twelfth most common class of neoplastic disease, and the eleventh most common cause of cancer-related death. About 90% of all leukaemia are diagnosed in adults (Mathers *et al*., 2001; LLS, 2009).

Although, the exact cause of leukaemia is still unknown, scientists suspected that viral, genetic, environmental or immunological factors may be involved (Tanner *et al*., 2001). There are two main categories of leukaemia: acute and chronic. Chronic leukaemia is primarily the disease of adults, with the exception of chronic myelogenous leukaemia which sparingly occur in children. In acute leukaemia, about 80% of Acute Lymphoblastic Leukaemia (ALL) occurs in children and Acute Myeloblastic Leukaemia (AML) is far more common in adults. While acute leukaemia is acutely fatal if untreated, the chronic leukaemia is relentlessly insidious and may only be diagnosed in the early stage during routine medical check-up (Devi *et al.,* 2000).

The incorporation of nutritional screening and comprehensive assessments is increasingly recognised as imperative in the development of standards for quality care in oncology (McMahon *et al*., 1998). Trace elements have been extensively studied over recent years to assess whether they have any modifying effects regarding the aetiology of cancer. Different researchers have tried to establish a relationship between trace elements and malignant diseases. Changes in blood levels of zinc and copper have been found in lymphoproliferative disorders as well as in breast, lung and gastrointestinal tumours (Oyama *et al*., 1994; Rosas *et al*., 1995; Jayadeep *et al*., 1997; Ferrigno *et al*., 1999; Arinola and Charles-Davies, 2008). Several studies have implicated oxidative stress in the development of several chronic diseases including cancer (Mehmet *et al*., 2006). Zinc is an important element in the preservation of immune resistance

*Corresponding author: Tel: +234 8023451520; E-mail: drarinolaog64@yahoo.com

and both Zn and Cu are required for numerous biochemical functions and for optimal activity of the immune system (Taysi *et al*., 2003). Copper (Cu) and Fe can catalyse the formation of the highly reactive hydroxyl radicals from H_2O_2 via the Haber-Weiss reaction and decompose lipid peroxides to peroxyl and alkoxyl radicals, which favours the propagation of lipid oxidation (Zago and Oteiza, 2001).

Nitric Oxide (NO) is a free radical and multifunctional molecule that is produced in many mammalian cells (Tsumori *et al*., 2002). Previous studies showed that NO has cytotoxic effects on human cell lines from patients with leukaemia or lymphoma (Kwon *et al*., 1991; Jiang *et al*., 1992; Magrinat *et al*., 1992; Dugas *et al*., 1996; Filep *et al*., 1996), thus, raising the possibility that a NO donor might serve as a chemotherapeutic agent for acute leukaemia.

Therefore, this study evaluated the levels of NO, some essential trace metals (Zn, Cu, Fe, and Se), Superoxide dismutase (SOD) activity and malondialdehyde (MDA) in Nigerians with acute leukaemia so as to define their roles in improving treatment or appropriate care in remission.

MATERIALS AND METHODS

Patients

Twenty five (25) consecutive newly diagnosed patients with acute leukaemia (14 ALL and 11 AML), with age range of 4 to 50 years were selected through constellation of clinical features, full blood count and bone marrow studies. The patients were attending clinic in the Department of Haematology, University College Hospital, Ibadan, Nigeria between March, 2008 and November, 2010. Twenty five age-matched healthy subjects were taken as control group. All participants consented to the study.

Biochemical Measurement

Five millilitres (5 ml) of venous blood was collected from each subject into heparinised bottles to obtain plasma after centrifugation for 5 minutes at 4000 x g. The plasma obtained was stored at -20^{0}C until analysed. For trace element assays, all the materials (glass and plastic) used were thoroughly cleaned with hot solution of nitric acid (20%, v/v) for 48 hours and rinsed five times with deionised water. Trace metals (Cu, Zn, Se, and Fe) levels were determined using Beck 200 Atomic Absorption Spectrophotometer (AAS) as described by Arinola and Charles-Davies, 2008). The method is based on the principle that atoms of the elements vaporised when aspirated into the AAS and absorb light of the same wavelength as that emitted by the element when in the excited state. The level of NO was determined with Griess reagents using spectrophotometric method (Laudanska *et al*., 1970). Superoxide Dismutase (SOD) and malondialdehyde (MDA) levels were determined using Enzyme Linked Immunosorbent assay (ELISA) kits (Cell Biolabs, Inc., USA) following the manufacturer's procedure.

Statistical Analysis

Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 15.0). Results were expressed as mean ± SD and student t-test was used to compare the means. A *p*-value <0.05 was considered statistically significant.

RESULTS

The mean levels of plasma Zn, Fe and Se were slightly elevated in leukaemia patients compared with controls. Also slightly lower level of plasma Cu was observed in leukaemia patients compared with the control. However, NO was significantly increased in leukaemia patients compared with controls ($p < 0.05$).

Table 1: Levels of Trace Elements, MDA, SOD Activity and NO in Leukaemia Patients and Controls

Measured Parameters	Leukaemia (n =25)	Control (n = 25)	t-values	*p*-values
Se (g/dl)	6.63 ± 1.93	5.91 ± 2.08	1.273	0.209
Cu (g/dl)	116.00 ± 26.02	119.58 ± 34.72	0.444	0.659
Fe (g/dl)	132.78 ± 33.00	122.84 ± 35.73	1.022	0.312
Zn (g/dl)	59.86 ± 9.01	56.72 ± 14.45	0.923	0.361
SOD(U/l)	4.99 ± 3.68	4.26 ± 2.74	0.792	0.432
MDA(pm/mg)	109.28 ± 39.51	97.44 ± 33.64	1.141	0.260
NO (µm/l)	33.12 ± 19.61	15.96 ± 10.69	3.84	0.0004

P is significant at p < 0.05 value (2-tailed)

DISCUSSION

Acute leukaemia (myeloblastic and lymphoblastic) is characterised by the rapid growth of abnormal clone of haemopoietic precursor cell within the bone marrow leading to undue accumulation in the bone marrow. This interferes with the normal process of haemopoiesis. The blasts also spill over into circulation and infiltrate various tissues and organs (LLS, 2009). The biological role of trace elements, especially serum copper and zinc, in different physiological and pathological conditions has been extensively investigated (Rosas *et al*., 1995; Arinola and Charles-Davies 2008).

Changes in copper and zinc concentrations have been found in lymphoproliferative disorders and also in ovarian, breast, lung and gastrointestinal tumours (Rosas *et al*., 1995; Arinola and Charles-Davies 2008). Statistically, no significant differences were observed in the mean plasma levels of all the parameters investigated in this study except NO in acute Leukaemia patients compared with controls. This observation corroborates earlier reports in acute leukaemia and other forms of malignancy (Magalova *et al*., 1999; Sgarbieri *et al*., 2006) but contradicts observation of Mehemet *et al*. (2006) in chronic lymphocytic leukaemia patients.

In the present study, levels of plasma Zn, Fe, Se, SOD and MDA were slightly elevated in acute leukaemia patients compared with controls whereas slightly lower level of plasma Cu was observed in leukaemia patients. The observed slightly raised level of Fe could be as a result of slightly elevated plasma level of superoxide activity which releases Fe^{2+} from ferritin thus, catalysing formation of hydroxyl radicals from H_2O_2 via the Haber-Weiss reaction. Also, this decomposes lipid peroxides to peroxyl and alkoxyl radicals that favour propagation of lipid oxidation (Murray *et al*., 2003). Superoxide dismutase activity and plasma MDA level were elevated in acute leukaemia patients compared with controls although the difference was not statistically significant. This shows that there is slightly increased lipid peroxidation in leukaemia patients possibly, as a result of oxidative stress due to free radical production.

Previous studies concluded that paediatric patients with ALL have higher oxidative stress but lower levels of antioxidants than healthy counterparts (Abou-Seif, 2000; Singh *et al.,* 2001; Phanphen *et al*., 2008). The implication of the present study is that intervention to increase antioxidant status in patients with ALL should be considered. Davi *et al*. (2000) reported that there were no significant differences in plasma malondialdehyde levels, red cell copper zinc superoxide dismutase (Cu-Zn SOD) and glutathione peroxidase (GSH-PX) activities in different types of leukaemia, suggesting that the changes are not specific to the type of leukaemia. A study showed that, the combination of phenolic antioxidant compounds (such as curcumin, carnosol, or quercetin) in conjunction with NOS inhibitors may be particularly valuable as a novel strategy for treating acute leukaemia (Kellner and Zunino, 2004).

Another study demonstrated that sodium nitroprusside (SNP), a NO donor, had cytotoxic effects on cells of some patients with Malignant Lymphoma (ML), Acute Myeloblastic Leukaemia (AML) or Chronic Myelomonocytic Leukaemia (CMMoL), but not with multiple myeloma (Tsumori *et al*., 2002). Also, elevated production of NO has been observed in B-cell chronic lymphocytic leukaemia, and appears to play a survival role in these types of cells (Zhao *et al*., 1998; Kolb, 2000). Therefore, significantly raised level of NO observed in the present study suggests that elevated nitric oxide might be a potential chemotherapeutic target for the treatment of acute leukaemia. In general, the results of this study suggest that oxidative stress is a feature of acute leukaemia and NO may be considered in the management of acute leukaemia patients.

REFERENCES

Abou-Seif MA, Rabia A and Nasr M (2000). Antioxidant Status, Erythrocyte Membrane Lipid Peroxidation and Osmotic Fragility in Malignant Lymphoma Patients. *Clin Chem lab Med*. **38**: 737-742

Arinola OO and Charles-Davies MA (2008). The Plasma Levels of Trace Elements in Nigerian Males with Different PSA Values. *Malaysian J Med Sci*. **15** (2): 33-36

Devi GS, Prasad MH, Saraswathi I, Raghu D, Rao DN and Reddy PP. (2000). Free Radicals Antioxidant Enzymes and Lipid Peroxidation in Different Types of Leukaemia. *Clin Chem Acta*. **293**(1-2): 53-62

Dugas N, Mossalayi, MD, Calenda A, Léotard A, Bécherel P, Mentz F, Ouaaz F, Arock M, Debré P, Donald J and Dugas B (1996). Role of Nitric Oxide in the Anti-tumoral Effect of Retinoic acid and 1, 2, 5-

dihydroxyvitamine D3 on Human Promyelocytic Leukemic Cells. *Blood*. **88**:3528–3534

Ferrigno D, Buccheri G and Camilla T (1999). Serum Copper and Zinc Content in Non-small Cell Lung Cancer: Abnormalities and Clinical Correlates. *Monaldi Arch Chest Dis*. **54**(3):204-208

Filep JG, Baron C, Lachances S, Perreault C and Chan JSD (1996). Involvement of Nitric Oxide in Target-cell Lysis and DNA Fragmentation Induced by Murine Natural Killer Cells. *Blood*. **87**: 5136–5143

Jayadeep A, Raveendran Pillai K and Kannan S (1997). Serum Levels of Copper, Zinc, Iron and Ceruplasmin in Oral Leukoplakia and Squamous Cell Carcinoma. *J Exp Clin Cancer Res*.**16** (3):295-300

Jiang H, Stewart CA, Fast DJ and Leu RW (1992). Tumor Target-derived Soluble Factor Synergies with IFN-Gamma and IL-2 to Activate Macrophages for Tumor Necrosis Factor and Nitric Oxide Production to Mediate Cytotoxicity of the Same Target. *J Immunol.* **149**: 2137–2146

Kellner C and Zunino SJ (2004). Nitric Oxide is Synthesized in Acute Leukaemia Cells after Exposure to Phenolic Antioxidants and Initially Protects against Mitochondrial Membrane Depolarization. *Cancer Lett*. **44** (215): 43–52

Kolb JP (2000). Mechanisms Involved in the Pro- and Anti-apoptotic Role of NO in Human Leukaemia. *Leukaemia*. **14**: 1685–1694

Kwon NS, Stuehr DJ and Nathan CF (1991). Inhibition of Tumor Cell Ribonucleotide Reductase by Macrophage-derived Nitric Oxide. *J Exp Med.* **174**: 761–767

Laudanska E, Gwozdz A, Brudel G and Rajchert J (1970). Evaluation of Griess Reagent and TTC Tests in Obstetrics. *Ginekol Pol*. **41**:857-862

Leukaemia and Lymphoma Society (2009). "Leukaemia Facts and Statistics: *http://www.lls.org/diseaseinformation/getinformationsupport/factstatistics/*

Magalova T, Bella V, Brtkova A, Beno I, Kudlackova M and Volkovova K (1999). Copper, Zinc and Super Oxide Dismutase in Precancerous Bening Diseases and Gastric, Colorectal and Breast Cancer. *Neoplasma*. **46**(2):100-104

Magrinat G, Mason SN, Shami PJ and Weinberg JB (1992). Nitric Oxide Modulation of Human Leukaemia Cell Differentiation and Gene Expression. *Blood*. **80**: 1880–1884

Mathers CD, Boschi-Pinto C, Lopez AD and Murray CJL (2001). Cancer Incidence, Mortality and Survival by Site for 14 Regions of the World. *Global Programme on Evidence for Health Policy Discussion Paper No. 13*: World Health Organization

McMahon K, Decker G and Ottery FD (1998). Integrating Proactive Nutritional Assessment in Clinical Practices to Prevent Complications and Cost. *Semin Oncol*. **25**:20-27

Mehmet G, Hasan K, Ilhami G, Fuat E, Kerim C, Mustafa K and Adnan Y (2006). Oxidase Activity Of Ceruloplasmin And Some Acute Phase Reactant And Trace Element Concentrations In Serum Of Patients With Chronic Lymphocytic Leukaemia. *SMJ*. **52**(1): 24-27

Oyama T, Matsuno K, Kawamoto T, Mitsudomi T, Shirakusa T and Kodama Y (1994). Efficiency of Serum Copper/Zinc Ratio for Differential Diagnosis of Patients With and Without Lung cancer. *Biol Trace Elem Res*. **42** (2):115-127

Phanphen P, Nalinee C, Samart P, Rachaneekorn WK and Umaporn S (2008). Antioxidant Status and Lipid Peroxidation in Pediatric Patients with Acute Lymphoblastic Leukaemia. *FASEB J.***22**:890-899

Rosas R, Poo JL, Montemayor A, Isoard F, Majluf A and Labardini J (1995). Utilidad De la Relación Cobre/zinc en Pacientes Con Linfoma o Leucemias Aguda o Crónica. [Utility of the Copper/Zinc Ratio in Patients with Lymphoma or Acute or Chronic Leukaemias]. *Rev Invest Clin*. **47**(6):447-452

Sgarbieri UR, Fisberg M, Tone LG and Latorre MD (2006). Nutritional Assessment and Serum Zinc and Copper Concentration among Children with Acute Lymphocytic Leukaemia: A Longitudinal Study. *Sao Paulo Med J*. **124**(6):316-320

Singh V, Ghalaut PS, Kharb S and Singh GP (2001). Plasma Concentrations of Lipid Peroxidation Products in Children with Acute Leukaemia. *Indian J*

Med Sci. **55**(4): 215-217

Tanner SM, Austin JL, Gustavo L and Baal C (2001). The Human Member of a Novel Mammalian Neuroectoderm Gene Lineage is Implicated in Hematopoiesis and Acute Leukaemia. *Proc Natl Acad Sci*. **98**: 13901-13906

Taysi S, Akcay F, Uslu C, Dogru Y and Gulcin I (2003). Trace Elements and Some Extracellular Antioxidant Proteins Levels in Serum of Patients with Laryngeal Cancer. *Biol Trace Elem Res*. **91**(1): 11-8

Tsumori M, Tanaka J, Koshimura K, Kawaguchi M, Murakami Y and Kato Y (2002). Cytotoxic Effect of Nitric Oxide on Human Haematological Malignant Cells. *Acta Biochimica Polonica*. **49** (1) 139–144

Zago MP and Oteiza PI (2001). The Antioxidant Properties of Zinc: Interactions with Iron and Antioxidants. *Free Rad Biol Med*. **31**(2): 266-74

Zhao H, Dugas N, Mathiot C, Delmer A, Dugas B, Sigaux F and Kolb JP (1998). B-cell Chronic Lymphocytic Leukaemia Cells Express a Functional Inducible Nitiric Oxide Synthase Displaying Antiapoptotic Activity. *Blood*. **92**: 1031–1043