Original Article

Antioxidant Levels of Acute Leukaemia Patients in Nigeria

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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 a 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...
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°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>
<thead>
<tr>
<th>Measured Parameters</th>
<th>Leukaemia (n =25)</th>
<th>Control (n = 25)</th>
<th>t-values</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se (g/dl)</td>
<td>6.63 ± 1.93</td>
<td>5.91 ± 2.08</td>
<td>1.273</td>
<td>0.209</td>
</tr>
<tr>
<td>Cu (g/dl)</td>
<td>116.00 ± 26.02</td>
<td>119.58 ± 34.72</td>
<td>0.444</td>
<td>0.659</td>
</tr>
<tr>
<td>Fe (g/dl)</td>
<td>123.78 ± 33.00</td>
<td>122.84 ± 35.73</td>
<td>1.022</td>
<td>0.312</td>
</tr>
<tr>
<td>Zn (g/dl)</td>
<td>59.86 ± 9.01</td>
<td>56.72 ± 14.45</td>
<td>0.923</td>
<td>0.361</td>
</tr>
<tr>
<td>SOD(U/l)</td>
<td>4.99 ± 3.68</td>
<td>4.26 ± 2.74</td>
<td>0.792</td>
<td>0.432</td>
</tr>
<tr>
<td>MDA (pm/mg)</td>
<td>109.28 ± 39.51</td>
<td>97.44 ± 33.64</td>
<td>1.141</td>
<td>0.260</td>
</tr>
<tr>
<td>NO (µm/l)</td>
<td>33.12 ± 19.61</td>
<td>15.96 ± 10.69</td>
<td>3.84</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

*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$_2$O$_2$ 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.

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