



LEAD TOXICITY AND TOTAL ANTIOXIDANT DEPLETION IN CHILDREN WITH ACUTE LEUKEMIA IN SOUTH WESTERN NIGERIA

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ABSTRACT

Background: Acute leukemia is one of the major Haemetological malignances that has increased malignancy related morbidity and mortality worldwide. The role of toxic metals as well as oxidant-antioxidant capacity in this condition has not been fully evaluated in Nigeria. **Methodology:** Twenty-five subjects (between 2 and 16 years) newly diagnosed with acute leukemia and 25 age and sex matched apparently healthy children were recruited as test subjects and controls respectively for the study. Serum trace elements (Cd, Pb, Hg, and As) of both subjects and controls were determined with atomic absorption spectrophotometer. The total antioxidant potential (TAP) was determined using Ferric reducing/antioxidant power (FRAP) assay and malondialdehyde (MDA) was measured to evaluate the extent of oxidant damage to membrane lipids using method of Botsuglou et al. **Results:** There was a significant increase in mean serum level of Lead (Pb) in patients with leukemia compared with controls; 15.74 ± 2.60 vs 13.80 ± 2.30 ($p < 0.05$). There was no significant difference in mean serum levels of Cd, Hg, and As ($p > 0.05$) between the study groups. However, there was a significant reduction in the total antioxidant potential (TAP) in patients with leukemia when compared with healthy controls (452.24 ± 88.00 vs 633.70 ± 101.06 ; $P < 0.05$). Malondialdehyde did not change significantly in subjects and controls ($P > 0.05$). A significant fairly strong negative correlation was observed between TAP and Pb ($r = 0.407$, $p = 0.044$). **Conclusion:** Elevated serum Pb level and its strong negative correlation with TAP suggest a role of Lead toxicity in the pathogenesis of acute leukemia in Nigeria.

KEYWORDS: Toxic metals, Total Antioxidant potential, Acute Leukemia.

INTRODUCTION

Childhood leukemia is said to be the most common cause of malignancy under the age of 15.^[1] A study from Northern Nigeria reported that acute leukemia represents 16.9% of all childhood malignancies². The association of acute leukemia in childhood with environmental factors has been established in different parts of the world. Trace metals, some of which have been classified as heavy metals, depending on their relative density to water have been variously identified as important environmental contaminants in the pathogenesis of many disorders, including acute leukemia^[3-7]

It has been stated in recent years that, there have been an increasing ecological and public health concern associated with environmental contamination by these metals.^[8] Human exposure to trace metal pollution has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications.^[8] Water contaminants (especially Cadmium and Arsenic) have been linked with an increased risk of childhood leukemia in a study from Canada.^[5] Also, increased Copper, Lead and Cadmium

has been linked with acute leukemia cases in Turkey.^[7] In Nigeria however, there is paucity of data relating to the role of toxic trace metals as well as status of antioxidant capacity in acute leukemia. This study was therefore designed to determine the serum levels of selected heavy trace metals which include Cadmium (Cd), Lead (Pb), Mercury (Hg) and Arsenic (As); malondialdehyde (MDA, product of oxidant damage to membrane lipids) and total antioxidant potential (TAP) in newly diagnosed acute leukemia in children. It is our hope that our findings will further contribute to the pool of available knowledge on the possible association of heavy metal toxicity in the development of childhood acute leukemia especially in Nigerian subjects.

MATERIALS AND METHODS

The setting was the Haematology out-patient clinic of University College, Ibadan, Oyo state, Nigeria. Twenty five newly diagnosed children with acute leukemia between the ages of 2 and 16 years were successively recruited after an informed consent from the parents/guardians. The diagnosis was made by

examination of their blood films as well as establishment of clinical signs and symptoms by Haematologists in the Clinic. Another 25 apparently healthy age and sex matched children were recruited from a primary school in the same town after an informed consent both from the school authority and the parents/guardians to serve as controls. The blood films from these controls were also examined to exclude possibility of undiagnosed leukemia. Children with already established leukemia on treatment were excluded.

Five millilitres (5mls) of venous blood sample was collected from each subject and control using aseptic procedure of phlebotomy. Three millilitres of blood was dispensed into acid-washed plain glass specimen bottle for trace metal analysis while the remaining was dispensed into lithium heparin specimen bottle for the estimation of total antioxidant potential (TAP) and malondialdehyde (MDA). Samples in the acid washed plain specimen bottles were allowed to clot and retract for 1 hour and then centrifuged at 3000rpm for five minutes along with those in lithium heparin specimen bottles. Serum and plasma were harvested and transferred into acid washed plain specimen bottles and non-acid washed plain specimen bottles respectively and stored frozen at -20°C prior to analysis.

Serum Cadmium (Cd), Lead (Pb), Mercury (Hg) and Arsenic (As), were estimated by flame atomic absorption

spectrophotometry using direct method as described by Kaneko *et al*, 1999.^[9] Total Antioxidant Potential (TAP) was determined using ferric reducing/antioxidant power (FRAP) assay as described by Benzie *et al*, 1996.^[10] Level of lipid peroxidation was determined by measuring the malondialdehyde (MDA) level using a method as described by Botsoglou *et al*, 1994.^[11]

RESULTS

The mean values and standard deviation of serum level of measured heavy metals (Cd, Pb, Hg, As), total antioxidant potential (TAP) and lipid peroxidation product (MDA) in Leukemia and control were presented in table 1. There were no significant differences in the serum levels of Cd, Hg and As in patients with leukemia when compared with controls. However, the serum level of Lead is significantly higher than that of control 15.74 ± 2.40 vs 13.80 ± 2.30 ($p < 0.05$). The total antioxidant potential (TAP) of the control group was significantly higher than in leukemia patients (633.70 ± 101.06 vs 452.24 ± 88.00).

Table 2 shows significant negative correlation between serum Pb and TAP ($r = -0.407$ and $p = 0.044$) in the leukemia subjects. We also observed a significant positive correlation between Pb and Cd in leukemia patients ($r = 0.727$, $p = 0.000$). No significant correlations were found in controls.

Table 1: Comparison of Mean $\pm$ 2SD of Serum Biochemical Parameters between the study groups

Parameter	Leukemia N=25	Control (X $\pm$ S.D) N = 25	t-test	P-value
Cd ($\mu\text{g/dl}$)	17.64 ± 2.60	18.74 ± 2.07	1.612	0.114
Pb ($\mu\text{g/dl}$)	15.74 ± 2.40	13.80 ± 2.30	2.933	0.005*
Hg ($\mu\text{g/dl}$)	7.20 ± 2.30	7.91 ± 1.93	1.161	0.252
As ($\mu\text{g/dl}$)	9.36 ± 1.41	9.87 ± 1.25	1.318	0.194
TAP ($\mu\text{mol Trolox equiv/L}$)	452.24 ± 88.00	633.70 ± 101.06	6.649	0.000*
MDA ($\mu\text{mol/dl}$)	11.11 ± 35.52	9.74 ± 33.64	1.394	0.170

*Significant at $p < 0.05$

Table 2: Correlation of Measured Biochemical Parameters in Subjects with Leukemia

Parameter	Cd	Pb	Hg	As	TAP	MDA
Cd r-values	1	0.727	0.286	-0.031	-0.324	-0.013
P-values		0.000*	0.166	0.882	0.114	0.950
Pb r-value	0.727	1	0.287	-0.355	-0.407	0.102
p-values	0.000*		0.164	0.821	0.044*	0.627
Hg r-value	0.286	0.287	1	0.003	-0.254	-0.123
p-values	0.166	0.164		0.990	0.220	0.560
As r-value	-0.031	-0.355	0.003	1	0.376	0.037
p-values	0.882	0.081	0.990		0.064	0.861
TAP r-value	-0.324	-0.407	-0.254	0.376	1	0.040
p-values	0.114	0.044*	0.220	0.064		0.851
MDA r-value	-0.013	-0.123	-0.123	0.037	-0.040	1
p-values	0.950	0.560	0.560	0.861	0.851	

r- values=Pearson's correlation

p- values=significant values

*correlation is significant at $p < 0.05$ level (2-tailed)

DISCUSSION

The International Agency for Research on Cancer has classified inorganic Lead as a probable carcinogen^[12], therefore, significant increase in serum levels of Pb in our leukemia patients strongly suggest a role for Pb poisoning in pathogenesis of acute leukemia in this environment. Our findings are similar to the findings of study carried out in Turkey.^[7] They reported an elevated serum Lead, Copper and Cadmium in acute leukemia cases. Another study from Canada^[5] implicated elevated Cadmium and Arsenic in childhood leukemia. Contrary to these findings, our study observed Cadmium and Arsenic not significantly elevated in patients with leukemia. Only Lead was found to be significantly higher in acute leukemia cases as observed in our study. The source of lead contaminant has always been through environmental pollution.

Although, the use of lead in gasoline for road vehicles was phased out in 1980s and early 1990s, it is still being used in some petrochemical industries and this remains a major source of environmental Pb poisoning in Nigeria. Lead in the exhaust also contaminate the soil near roads through which our food crops, vegetable and drinking water becomes contaminated. Apart from Lead in the gasoline, another important source of lead contamination is via inhalation of dust from Lead contaminated deteriorated house hold paints. These two important sources are of particular importance in pregnant women because lead absorbed during pregnancy is readily transferred to the foetus. Also, children who live in homes with deteriorated lead paint have been reported to have higher blood lead concentration. These facts had earlier been documented in the works of Heck et-al^[13] and Belson M et-al.^[14]

Significantly strong positive correlations between Cadmium and Lead in our leukemic subjects suggest a possible involvement of Cadmium or its symbiotic effect on Lead in acute leukemia. This hypothesis may deserve further exploration.

We also observed a significant reduction in the level of total antioxidant potential (TAP) in acute leukemia patients compared with the levels of normal non-leukemia control subjects. The reduction in total antioxidant potential suggested that some of these antioxidants have been used up during the process of oxidative stress in response to increased free radical generation. Previous studies have shown that reactive oxygen species (ROS) production and oxidative stress are involved in the pathogenesis of toxicity and carcinogenicity of metals such as Lead^[15,16], Cadmium^[17], Mercury^[18,19] and Chromium.^[20] Furthermore, the inverse correlation of plasma Lead level and total antioxidant potential found in our study further support the hypothesis of increased ROS production and oxidative stress in the pathogenesis of acute leukemia. Evidence from our study and earlier published report strongly implicated the involvement of

free radical and oxidative stress pathways in the pathogenesis of acute leukemia. Our finding of reduced total antioxidant potential in this study population may suggest possible benefit of antioxidant supplementation in acute leukemia.

Increased MDA (malondialdehyde) level in the leukemic patients found in this study, though not statistically significant, further suggests evidence for an increase in lipid peroxidation secondary to increased production of ROS. This may also suggest why antioxidants are used up.

CONCLUSION

Lead poisoning and reduced total antioxidant potential may be involved in the pathogenesis of acute leukemia. A possible involvement of oxidative stress through generation of ROS and resultant increase in lipid peroxidation product are suggested in the pathogenesis of acute leukemia. An exploration of this pathway and possibility of antioxidant supplementation in the prevention and management of acute leukemia may be further recommended.

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