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ORIGINAL ARTICLE

Impaired Antioxidant-Defence Status in Nigerian Children with Elevated Blood Lead Levels: A Possible Predisposing Factor to Chronic Diseases

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Abstract:

Background: Lead, a prime environmental toxicant and multi-organ poison, exerts its toxicity through interaction with essential metals and the generation of free radicals. *Aim and Objectives*: To explore and elucidate possible interaction dynamics between lead and the antioxidant-defence status in apparently healthy children with Elevated Blood Lead Level (EBLL) in Ibadan, South-West, Nigeria. *Material and Methods:* Three-hundred-and-nine apparently healthy, public primary school children were grouped into 169 children with EBLL (BLL > 5µg/dL) and 140 control (BLL⩽ 5µg/dL). Blood lead (Pb), plasma enzymatic antioxidants [Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activities], antioxidant metals [Copper (Cu), Zinc (Zn), Selenium (Se)], and oxidative stress parameters [Total Antioxidant Potential (TAP), Total Plasma Peroxide (TPP) and Oxidative Stress Index (OSI)] were determined. *Results:* Levels of Pb, Zn, Se, TPP and OSI increased while SOD, GPx and TAP decreased in children with EBLL compared with control ($p<0.05$). No difference was observed in Cu levels between the two groups ($p > 0.05$). Lead associated positively with Se, TPP and OSI ($p<0.05$), negatively with TAP, SOD and GPx ($p<0.05$) but showed no association with Zn and Cu ($p>0.05$). *Conclusion:* There was impaired antioxidant-defence status in children with EBLLs which may predispose them to chronic diseases if unresolved.

Keywords: Antioxidants, Essential Metals, Lead Exposure, Free Radicals, Oxidative Stress Index, Total Antioxidant Potential, Total Plasma Peroxide

Introduction:

Lead (Pb), a prime ubiquitous environmental toxicant, present in contaminated water, air, food, dust and consumer products, is a multi-organ poison which induces biochemical, behavioural and psychological disturbances in humans [1-3]. Lead exposure constitutes a serious health concern in children because they have high intestinal Pb absorption, relatively ineffective detoxification and elimination mechanisms and special tendency to nutritional deficiencies which can exacerbate lead toxicity [4]. Some possible mechanisms by which Pb may cause toxicity have been reported [4]. However, the underlying basic mechanisms involved include interactions with essential metals and increased generation of free radicals; which are highly reactive, unstable species that can

destroy vital macromolecules leading to cell damage and homeostatic disruption, if uncontrolled [5-6]. Antioxidants are relatively stable compounds that reduce or neutralise the effects of free radicals under normal physiological conditions [7]. However, lead exposure can create aberrations through increased generation of free radicals which may overwhelm the antioxidant system and result to oxidative stress - a precursor of organ damage [7].

The antioxidant-defence system is made up of complex array of antioxidants which include enzymes and essential metals [8]. Front-line endogenous enzymatic antioxidants include Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) [9]. SOD scavanges superoxide free radical and converts it to hydrogen peroxide. GPx constantly and efficiently removes the load of hydrogen peroxide and enhances the availability of glutathione, one of the most abundant intrinsic antioxidant [9]. The essential metals, such as Zinc (Zn), Copper (Cu) and Selenium (Se), orchestrate several key physiological functions, which may include functioning as co-factors for enzymatic antioxidants [9]. They may also interfere with absorption, post-exposure tissue distribution, deposition and excretion processes of Pb [5].

Reports have shown that the numerous antioxidants in plasma and the possible interaction among them could make the assessment of each antioxidant less representative of all the antioxidants in plasma [10-11]. Hence, assessing the Total Antioxidant Potential (TAP), which represents the synergic and redox interaction among the different antioxidants in plasma, and, the Total Plasma Peroxide (TPP), which may be considered a reflection of free radical concentrations in plasma, become imperative in the assessment of antioxidant status [10-11].

Conflicting reports exist for the interaction between blood Pb, a commonly used biomarker of Pb exposure [12] and antioxidants in adults occupationally exposed to Pb [7,8,13]. However, this concept has been poorly explored in children particularly in developing countries such as Nigeria, with prevailing nutritional deficits [14], high environmental chemical burden [15-16] and poor/unimplemented environmental regulations [17]. Therefore, the aim of this study was to explore and elucidate the possible interaction dynamics between blood Pb and the antioxidant-defence status (selected antioxidant metals, antioxidant enzymes and oxidative stress parameters), in apparently healthy children with Elevated Blood Lead Level (EBLL) in Ibadan, South-West, Nigeria.

Material and Methods:

Study Population:

This study was cross-sectional and involved recruitment of 309 apparently healthy, public primary school children (aged 8 - 10 years) who had been resident in Ibadan, Oyo State, South-West, Nigeria, for 5 years and above. The selection of 8 public primary schools for this study was based on multistage random sampling technique while the recruitment of the children from the schools was based on parental consent, verbal assent and physical presence on the day of sampling as reported in Nwobi *et al.*, 2019 [2].

The participating children were grouped into two; 169 children (83 males and 86 females) with EBLL and 140 control (71 males and 69 females). EBLL was defined as BLL > 5µg/dL while

controls where children with acceptable BLL defined as BLL ⩽ 5µg/dL; recommended by the US Centre for Disease Control and Prevention as cut-off for childhood BLL [18].

Sample size was estimated using the standard formula;

Sample size (N)= Z^2 p (1-p)/D^2 [19]

Where; Z= 1.96 (the standard normal deviate at 95% confidence level)

p = prevalence of Nigerian children with EBLL = 11.4% [20]

D= margin of unacceptable error or measure of precision = 0.05

Sample size (N) = $(1.96)^2$ x 0.114(1-0.114) / 0.05 x 0.05 = 155.

Inclusion criteria involved apparently healthy public primary school children with signed informed parental consent and verbal assent while, exclusion criteria involved children with evidence of lead exposure requiring chelation therapy, anaemia, malnutrition, acute/chronic infection, stress, intake of mineral supplements or any obvious pathology [2].

Ethical Consideration:

This study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Research Ethics Committee, Nigeria as well as the Ministry of Education, Oyo State, Nigeria. The parents/guardian of the children received oral and written information about the study protocol in both English language and their local dialect. Thereafter, signed informed consent was obtained from those that agreed that their children should participate in the study. Verbal assent was also obtained from the participating children.

Blood Sampling and Biochemical Analysis:

Blood sampling of the participants was carried out on site during regular school days between 8 and 10 a.m. before classes commenced. About 10 mL of non-fasting venous blood samples were collected from the antecubital vein of each participant by a trained paediatric phlebotomist into heparinised tubes and centrifuged at 3000 ×g for 10 min to obtain plasma which was aliquoted into acid washed plain bottles (10% (v/v) HNO_3 to remove contaminants), and subsequently stored frozen at −80°C until use. Analyses of blood Pb and plasma essential metals; Cu, Zn and Se were determined with graphite furnace atomic absorption spectrometer Perkin-Elmer A Analyst 800 with Zeeman-effect background correction (Norwalk, U.S.A), as already reported [2, 5]. Plasma SOD and GPx activities were measured by Enzyme Linked Immunosorbent Assay (ELISA) kit purchased from Cell Biolabs Inc. San Diego, CA 92126, and Biovendor research and diagnostic products, Asheville, SeverníKarolína 28806, US respectively. TAP was determined using the Ferric Reducing Antioxidant Power (FRAP) assay according to the method of Harma *et al*. (2003) [21]. Total Plasma Peroxide concentrations, were determined using the FOX2 method [22]. Oxidative Stress Index (OSI) was calculated as the ratio of TPP to TAP [21]. All standards and reagents used were of analytical grade and of highest purity. Standard precautions were taken to avoid external contamination during collection, storage and processing of samples. Results were only acceptable when data obtained fell within expected quality control samples (X ± 2SD).

Statistical Analysis:

Statistical Package for the Social Sciences (SPSS) statistical software program version 21.0 (SPSS Inc, Chicago, IL) was used for the statistical analysis. Values were assessed for normality by checking for skewness. Results were expressed as mean ± SD. Independent sample t-test was used to determine differences between children with EBLL and control. Pearson's product moment correlation analysis was used to evaluate the relationship between blood lead (independent variable) and Cu, Zn, Se, SOD, GPx, TAP, TPP and OSI (dependent variables). All tests were 2-tailed and p value < 0.05 was statistically significant

Results:

The general characteristics; age, anthropometric indices, albumin and haematocrit levels in children with EBLL and control did not show any significant difference ($p > 0.05$) (Table 1), implying that they were not confounders to the study. Levels of blood Pb, plasma Zn, Se, TPP and OSI were significantly higher in children with EBLL compared with control ($p < 0.05$) (Table 2). In contrast, TAP, SOD and GPx were significantly lower in children with EBLL compared with the control ($p < 0.05$) (Table 2). No significant difference was observed in the levels of Cu between the two groups ($p > 0.05$) (Table 2). Blood Pb had significant positive correlation with Se ($r = 0.287$; $p < 0.001$), TPP ($r = 0.658$; $p = 0.026$) and OSI ($r = 0.762$; $p = 0.010$) but significant negative correlation with TAP ($r = -0.554$; $p = 0.030$), SOD ($r = -0.534$; $p = 0.022$) and GPx ($r = -0.479$; $p = 0.035$). However, Pb did not show any significant correlation with Zn and Cu ($p > 0.05$) (Table 3).

Table 1: General Characteristics of Children with EBLL and Control

Indices	Participants		t	*P*
	Control (N = 140)	EBLL (N = 169)		
Age (years)	8.9 ± 1.5	8.6±1.6	-1.711	0.088
Weight (kg)	24.5 ± 4.8	23.8 ± 4.8	-1.015	0.311
Height (m)	1.37 ± 10.1	1.3 ± 9.2	-1.285	0.200
BMI (kg/m^2)	15.3 ± 1.8	15.1 ± 1.5	-0.539	0.590
Albumin (g/dL)	4.6 ± 0.4	4.5 ± 0.4	-0.941	0.347
Haematocrit (%)	36.7 ± 2.5	36.6 ± 3.3	-0.867	0.387

Results are presented as mean ± standard deviation, EBLL = Elevated blood lead level, Control = Acceptable blood lead level, BMI = body mass index

Table 2: Levels of Lead, Antioxidant Metals, Antioxidant Enzymes and Oxidative Stress Parameters in Children with EBLL and Control

Variables	Participants		t	*P*
	Control (N = 140)	EBLL (N = 169)		
Pb (µmol/L)	0.2 ± 0.3	0.4 ± 0.1	17.133	<0.001*
Se (µmol/L)	1.0 ± 0.2	1.2 ± 0.4	5.271	<0.001*
Zn (µmol/L)	10.7 ± 2.1	11.4 ±2.5	2.170	0.031*
Cu (µmol/L)	21.4 ± 5.2	22.4 ± 4.7	1.543	0.124
SOD (U/mL)	126.2±17.4	89.5±10.6	-22.790	<0.001*
GPx (U/mL)	3.7±0.5	1.87±0.3	-41.321	<0.001*
TPP (µmolH_2O_2/L)	9.1±1.5	15.1±4.2	16.157	<0.001*
TAP (µmolTroloxequiv/L)	960.9±121.5	893.6±123.0	-4.813	<0.001*
OSI	0.01±0.0	0.02±0.01	15.562	<0.001*

*Results are presented as mean ± standard deviation, EBLL = Elevated blood lead level, Control = Acceptable blood lead level, *= Significant at p < 0.05 (2- tailed), Pb = Lead, Se = selenium, Zn = Zinc, Cu = Copper, TPP = Total Plasma Peroxide, TAP = Total Antioxidant Potential, OSI = Oxidative Stress Index.*

Table 3: Correlation of Lead with Antioxidant Metals, Antioxidant Enzymes and Oxidative Stress Parameters in Children with EBLL

Correlating Pair Variables	Pb (µmol/L)	
	r	*P*
Zn (µmol/L)	0.043	0.450
Cu (µmol/L)	0.060	0.295
Se (µmol/L)	0.287	<0.001*
SOD (U/mL)	-0.534	0.022*
GPx (U/mL)	-0.479	0.035*
TPP (µmolH_2O_2/L)	0.658	0.026*
TAP (µmolTroloxequiv/L)	-0.554	0.030*
OSI	0.762	0.010*

** = Significant at p < 0.05,Pb = Lead, Zn = Zinc, Cu = Copper, Se = selenium, TPP = Total Plasma Peroxide, TAP = Total Antioxidant Potential, OSI = Oxidative Stress Index*

Discussion:

Lead exposure, commonly indicated by elevated blood lead level [18], remains a major environmental problem particularly in developing countries such as Nigeria, where it causes about 7% to 25% of the burden of diseases in children [23]. This study explored the possible involvement and interaction of blood lead with selected antioxidant enzymes, antioxidant metals and oxidative stress parameters in apparently healthy Nigerian children with elevated blood lead levels.

The observed decreased activities of front-line endogenous enzymatic antioxidants - SOD and GPx in children with EBLL and their inverse relationship with Pb could be a reflection of impaired activities of these enzymes as they possibly got consumed in the process of neutralising or reducing the increased free radicals possibly generated by Pb. This observation may be explained by the fact that when Pb interacts with SOD and GPx, it binds to the thiol groups in the active sites of these enzymes leading to alteration in their structure, subsequent inactivation and decreased activity of these enzymes resulting to possible reduction in the level of glutathione, the most abundant intrinsic antioxidant. This finding is in consonance with other reports [9, 24, 25].

The essential metals, Cu, Zn and Se are known to form the prosthetic groups of CuZn-SOD and GPx respectively [8, 9]. There is room to speculate that the observed pattern of increase in the plasma levels of these metals in children with EBLL occurred as a result of the release of their ions from the active site of CuZn-SOD and GPx owing to their competitive displacement by Pb ions [26]. This observation may also be interpreted as an adaptive up-regulation of these metals in the plasma. Increased demand of these metals as co-factors for CuZn-SOD and GPx enzymes and subsequent restoration of their antioxidant activities may have necessitated increase in the absorption of these essential metals for the mitigation of lead toxicity. Reports have shown that Cu, Zn and Se, beyond acting as antioxidants, can also interfere with the absorption, post-exposure tissue distribution, deposition and excretion processes of Pb [5-6].

In this study, the increased TPP and its positive relationship with Pb, observed in children with EBLL, may be interpreted as an indication of increased free radical generation by Pb [6-8]. Total antioxidant potential does not represent a single antioxidant but the interaction between free radicals and all the antioxidants [10-11]. The observed decreased TAP in children with EBLL and its inverse relationship with Pb could be a reflection of the compromised antioxidant status in the children with EBLL, which led to oxidative stress [7]. This view is supported by increased OSI coupled with its positive relationship with Pb in these children.

Oxidative stress, resulting from impaired anti-oxidant status, has been linked to damage to vital biomolecules such as lipid membranes, proteins and nucleic acids and is considered a precursor of damage to the organs of the body [8]. This condition has been reported to increase the risk of susceptibility to chronic diseases such as diabetes, cancer, hypertension, neurodegenerative, renal and reproductive diseases in adults [8]. Although, it may be difficult to identify the actual physiological and clinical significance of impaired antioxidant-defence status in children in this study, it cannot be overemphasised that if this condition is sustained or unresolved for a long time, its effects

could be potentially adverse. This, may render the children more susceptible to other stressors which, may most likely increase their vulnerability to diverse chronic diseases even before they become adults.

The World Health Organisation (WHO), in 2015, stated that the most practical way to mitigate lead exposure and its associated toxicity is to control and regulate potential sources of Pb contamination [27]. Yet, over the last decade, complete control and prevention over Pb exposure, its concomitant health implications and associated economic burden, have not been achieved, particularly in most developing countries [1, 3, 23]. However, it becomes imperative and appears safe to educate and increase the awareness of the general population on the importance of increased consumption of antioxidant-rich diets such as fruits, vegetables, grains and nuts [28]. This may be a promising support to enhance the health status and forestall the possible, devastating subtle complications associated with paediatric lead exposure and its toxicity, particularly in developing countries.

Conclusion:

This study showed decreased antioxidant enzyme activities and total antioxidant potential, increased levels of antioxidant metals, total plasma peroxide and oxidative stress in apparently healthy Nigerian children with elevated blood lead levels. These findings imply impaired antioxidant-defence status in these children which may predispose them to chronic diseases if unresolved.

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