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Inflammation, Oxidative Stress and Cobalt Deficiency in Acute Childhood Leukemia

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Abstract

Background: Despite increasing incidence of childhood leukemia in Nigeria, the pathophysiology remains poorly researched. The present study determined the levels of cobalt, C-reactive protein and markers of oxidative stress in children with acute leukemia.

Methods: Twenty-five (15 males, 10 females; age range: 3 to 14 years) newly diagnosed children with acute lymphoblastic leukemia were recruited for this study. Another twenty five (14 males, 11 females; age: 4-14 years) apparently healthy children without acute leukemia, septicemia or metabolic disorders at the time of the study served as controls. Plasma levels of cobalt, C-Reactive Protein (CRP), Total Plasma Peroxides (TPP), Malondialdehyde (MDA) and Total Antioxidant Potential (TAP) were determined in them using atomic absorption spectrophotometry, single radial immunodiffusion and spectrophotometry methods respectively. Level of Oxidative Stress Index (OSI) was calculated as percent ratio of TPP and TAP.

Results: The results showed significantly ($p<0.05$) higher plasma levels of TPP, OSI and MDA in acute leukemia children compared with controls. Plasma levels of TAP and cobalt reduced significantly ($p<0.05$) in children with acute leukemia compared with controls. The plasma level of OSI correlated significantly with TPP ($r=0.78$; $p<0.001$), TAP ($r= -0.73$; $p<0.001$), CRP ($r=0.64$; $p<0.001$) and cobalt ($r= -0.33$; $p=0.018$) in acute leukemia.

Conclusion: This study confirmed oxidative stress and inflammatory responses as features of childhood acute leukemia. The oxidative stress and cobalt deficiency could call for micronutrients supplementation as adjuvant therapy in the treatment of acute lymphoblastic leukemia.

Keywords: Acute leukemia; C-reactive protein; Oxidative stress; Micronutrient

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Introduction

Leukemia may be referred to as a consequence of chromosomal aberration, abnormal differentiation and pathological proliferation of the hematopoietic progenitor cells. The disease is characterized by abnormal increases in immature or dysfunctional white blood cells, coagulopathy, immune deficiency, anemia, fevers, chills, night sweats, weight loss and fatigue. The disease represents 2.9% of all cancers in the United States, and 30.4% of overall blood cancers [1]. It is clinically and pathologically subdivided into acute and chronic forms. Acute leukemia is the pediatric malignancy mostly found in children (0-14 years), while chronic leukemia majorly affects the adults. In 2000, approximately 256,000 children and adults around the world developed some forms of leukemia, and 209,000 died from it [2]. Although, about 90% of all leukemia's are diagnosed in adults, the incidence is increasing in the children [3]. The risk factors in children include maternal-fetal transmission of leukemia Wiernik [4], viruses such as human T-lymphotropic virus, radiation, carcinogenic substances, benzene and alkylating chemotherapy agents for previous malignancies, exposure to some petrochemicals, tobacco and hair dyes. The use of drugs to induce ovulation has also been implicated in a study conducted in France [5]. Boys are more likely to develop leukemia than girls, the Hispanics under the age of 20, are at the highest risk for leukemia [6].

Activated leukemia cells (usually by infectious processes) produce various molecules, including cytokines. In a study conducted by Pérez-Figueroa et al. [7], significantly higher levels of pro-inflammatory cytokines (IL-6 and TNF-α), T-cell-polarizing cytokines (i.e. IFN-γ, IL-12) and lower level of IL-13 (Th2 cytokines) were observed in patients with acute lymphoblastic leukemia without

infections. Previous studies show that increased metabolic activities of cancer cells enhance free radical production [8-10], especially the hydrogen peroxide (H_2O_2) that is more tumorigenic [11]. In a study conducted by Zhou et al. [12], oxidative damage was observed in patients with acute leukemia.

Cobalt, contained in a corrin ring of vitamin B12 is an essential trace metal linking the four pyrrole rings of cobalamin for effective synthesis of red blood cells [13]. An enzyme-mediated reduction of the cobalt occurs by cytoplasmic methylation to form methylcobalamin or *via* mitochondrial adenosylation to form adenosylcobalamin. Previous studies did not report any relationship of acute leukemia and plasma cobalt. But available information show that there are increases in the formation of cobalamin, retention and serum binding capacity in leukemic patients loaded with cobalt [14]. Normal level of cobalamin has been reported in undifferentiated acute leukemia [15]. The present study was designed to bridge this gap in knowledge by determining the status of plasma levels of cobalt, C-Reactive Protein (CRP), Total Plasma Peroxides (TPP), Malondialdehyde (MDA), Total Antioxidant Potential (TAP) and oxidative stress index.

Materials and Methods

Twenty five (15 males, 10 females; age: 3 to 14 years) newly diagnosed patients with acute lymphoblastic leukemia were recruited for this study. They were selected by the consultant hematologist in charge using clinical features, full blood count and bone marrow studies. Another twenty five (14 males, 11 females; age: 4-14 years) apparently healthy non-leukemic individuals served as controls. Verbal and written consents were obtained from parents of the children that participated in the study. Ethical clearance was obtained from the institutional ethical committee. 5 ml of blood sample was collected from every participant through venipuncture into lithium heparin bottles. The sample was centrifuged and the plasma separated into a plain bottle and stored at -20°C until ready for analysis.

Methods

Determination of MDA: Level of lipid peroxidation was determined in both human-milk and blood plasma by measuring the formation MDA using the method of Varshney and Kale [16]. The principle is based on the fact that Malondiahydehyde (MDA) produced from the peroxidation of membrane fatty acid reacts with the chromogenic reagent; 2-Thiobarbituric Acid (TBA) under acidic conditions to yield a pink-colored complex measured spectrophotometrically at 532 nm. 1,1,3,3-tetramethoxylpropane was used as standard.

Determination of TAP: TAP was determined in both human-milk and blood plasma using the Ferric Reducing/Antioxidant Power (FRAP) assay [17,18]. 1.5 ml of working pre-wormed (37°C) FRAP reagent (300 mM acetate buffer - pH-3.6, 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl and 20 mM FeCl3 at ratio 10:1:1) was vortex mixed with 50 µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv/L.

Determination of total plasma peroxide (TPP): Determination of TPP was based on the principle that ferrous-butylated hydroxytoluene-xylenol orange complex reacts with plasma hydrogen peroxide to form a color complex measured spectrophotometrically at 560 mm. H_2O_2 was used as standard. 1.8 ml of reagent 6 (F0X2) was mixed with 200 µ1 of plasma. This was incubated at room temperature for 30 min. 100 µMol H_2O_2 was used as standard. The mixture was centrifuged and the supernatant separated for reading at 560 nm [18].

Determination of oxidative stress index (OSI): OSI, an indicator of the degree of oxidative stress is the percent ratio of the TPP to the TAP [17].

Estimation of CRP: CRP was quantified by single radial immunodiffusion method [19]. A volume of an optimally diluted anti-CRP antiserum was mixed with noble agar and poured on glass plate. Wells of equal diameters were cut in the antibody-agar mixture. The wells were filled with test or standard sera. After incubation, the diameters of precipitin rings were measured using a Hyland viewer with a micrometer eyepiece.

Determination of Co: Cobalt was determined using Atomic Absorption Spectrophotometer (AAS) as described by Kaneko et al. [20]. The atomization of the element aspirated into the AAS results in the absorption of light of the same wavelength as that emitted by the element when in the excited state.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for windows, version 20. The data were expressed as Mean ± SD. Student (t) test was used for comparison of levels cobalt, CRP, TPP, MDA, TAP and OSI in children with acute leukemia and controls. Pearsonian correlation coefficient (r) was calculated. P values less than 0.05 were considered significant (Table 1).

Results

The plasma level of TPP was significantly ($p<0.001$) increased when compared with controls. OSI and MDA levels were significantly ($p<0.01$) higher in acute leukemia compared with controls. . There was a significantly ($p<0.001$) elevated level of CRP in acute leukemia, when compared with the controls. Plasma levels of TAP and cobalt were significantly (<0.001) lower in acute leukemia in children with acute leukemia compared with controls (Table 2). The plasma levels of OSI correlated significantly with TPP ($r=0.78$; $p<0.001$), TAP ($r= -0.73$; $p<0.001$), CRP ($r=0.64$; $p<0.001$) and cobalt ($r= -0.33$; $p=0.018$) in acute leukemia (Table 3).

Discussion

Cancer cells exhibit pathological proliferation and abnormal differentiation leading to deregulated metabolic activities. Susceptibility of the leukemic patients to infections enhances cellular activation. Excessive cellular activation and other factors might contribute to the increased levels of TPP, MDA and OSI observed in the acute leukemia children recruited for this study. This study seems to corroborate the report of Nieborowska-Skorska et al. [8] that alteration of mitochondrial membrane potential and abnormal flow of electron through the mitochondrial respiratory chain may lead to partial reduction of oxygen and generation of reactive oxygen species in the leukemic patients. In the studies conducted by Bianchi et al. [9] and Mandavilli et al. [10] it was observed that cells with mitochondrial DNA mutation generate higher levels of H_2O_2. Kumar et al. [21] observed increased free radical generation in typical cancer cells compared with normal cells. Zhou et al. [12] observed depressive effect of oxidative damage in patients with acute leukemia. In the study conducted by Rajeshwari et al. [22] and Saadaoui et al. [23] increased levels of oxidative stress were also reported in acute and chronic

Table 1: Demographic characteristics of Children with Leukemia and Controls.

	Controls (N=25)	Acute Leukemia (N=25)	t-values	p-value
Age (years)	9.52 ± 3.58	8.78 ± 4.24	1.77	0.092
Weight (Kg)	23.9 ± 5.09	11.65 ± 4.20	69.01	<0.001*
Height (M)	1.28 ± 0.15	1.06 ± 0.10	29.5	<0.001*
BMI (Kg/M²)	16.73 ± 1.85	10.51 ± 3.72	44.97	<0.001*

N: Sample size; *: Significantly different from controls

Table 2: Plasma Levels of Markers of Oxidative stress, CRP and Cobalt in Leukemia and Controls.

±	Controls (N=25)	Leukemia (N=25)	p-values
TPP (µM. H_2O_2/l)	10.3 ± 5.4	16.8 ± 6.8	<0.001*
TAP(µMol Trolox Equiv./l)	controls (N=25)	461.4 ± 96.9	<0.001*
OSI (%)	1.71 ± 0.96	3.9 ± 2.3	<0.010*
CRP (mg/l)	3.4 ± 1.85	22.0 ± 5.5	<0.001*
Cobalt (µg/dl)	10.24 ± 1.45	8.04 ± 2.11	<0.05*
MDA (mmol/ml)	2.45+1.32	10.9 ± 3.01	<0.001*

N: Sample size; *: Significantly different from controls

Table 3: Correlation between OSI, TPP, TAP, CRP and Cobalt Levels in Acute Leukemia (N=25).

Groups	r-values	p-values
OSI/TPP	0.782	<0.001*
OSI/TAP	-0.732	<0.001*
OSI/CRP	0.541	<0.001*
OSI/cobalt	-0.333	0.018*
OSI/MDA	0.178	>0.050

*: Significant correlation; N: number of acute leukemia patients

leukemic patients. Their findings therefore support the present study that shows higher level of TPP in children with acute leukemia. Increased level of TPP in the leukemic children might contribute to the significantly lower level of TAP, an index of total antioxidants in them. This study corroborates that of Naz et al. [24] who reported lower level of superoxide dismutase activity and oxidative stress in acute leukemia. In another study, Demir et al. [25] also observed lower levels of superoxide dismutase dependent elements (Zn and Mn) in acute leukemia. Elevated level of free radicals may lead to the gene mutation and also gelatinous transformation of the bone marrow and anemia; since red blood cells and haemopoietic stem cells are highly sensitive to deregulated accumulation of free radicals [26].

The elevated TPP level observed in our leukemia children could account for the increased abstraction of hydrogen atoms from the methylene groups (CH2 group) of long-chain polyunsaturated fatty acids (LC-PUFA) leading to increased lipid peroxidation [27]. Therefore, the elevated TPP observed in our study might contribute to the increased lipid peroxidation as demonstrated by the higher MDA observed in the acute leukemic children. Under normal physiological condition, MDA is quickly oxidized to acetate or malonate, and through Kreb's cycle, catabolized to carbon dioxide. But in conditions enhancing lipid peroxidation, excess MDA reacts favorably with different serum proteins and cell membrane components to form altered determinants [28]. It can also interact with DNA and inhibit the biosynthesis of the DNA, RNA, and proteins. The structure of MDA is similar to carcinogenic compounds like glyceraldehyde and β-propiolactone and its tumorigenicity has been postulated [28-30]. It may therefore be hypothesized in this study that the significantly higher level of MDA observed in our acute leukemia children could aggravate the DNA damage and carcinogenesis in them. This study corroborates the increased MDA levels observed in acute leukemia by Asfour et al. [31]. Our findings agree with other workers who reported significantly higher levels of MDA in various types of cancer such as breast, lung, oral, and cervical cancers and leukemia [31,32].

CRP is a marker of inflammation synthesized in the hepatocytes due to enhanced TNF-alpha and IL-6 release in response to tissue injury [33,34]. The physiological roles of CRP include opsonization, phagocytosis and lysis of invading organism like bacteria, viruses and prevent the establishment of a generalized systemic inflammation [35]. Higher level of CRP observed in our leukemic children could be due to increased cellular activation and release of pro-inflammatory cytokines. The higher CRP level in our acute leukemia patients could be a protective immunologic measure developed by the hepatocytes to reduce the risks associated with inflammation in them.

Cobalt is an essential metal linking the four pyrrole rings of cobalamin for effective synthesis of hem molecule. This element, in the presence of other factors has profound influence on erythropoiesis [13]. To our knowledge, this is the first study reporting significantly lower level of cobalt in acute leukemia. Diversion of the cobalt to other metabolic pathways other than cobalamin synthesis, and the usurpation during metabolic activities of leukemic cells could contribute to the lower level of cobalt in the acute leukemia children. The higher level of oxidative stress reported in these leukemia children could cause oxidation of cobalt and conversion of cyanocobalamin to a non-functional form as earlier stated by Hall and Malia [13]. A negative correlation observed between OSI and cobalt in this study seems to confirm the report of Hall and Malia [13]. This study may hypothesize that the oxidative modification of cobalt could account for its deficiency and anemia commonly reported in leukemia patients. Our result seems to contradict Demir et al. [25] who reported similar levels of Cobalt in the plasma of acute leukemia and non-leukemia individuals.

In conclusion, oxidative stress, increased CRP and cobalt deficiency may be features of acute leukemia. Children with acute leukemia may require antioxidants and micronutrients as adjuvant therapies.

Authors' Contributions

MOA designed the study, MOA, BSO, AAA, SOA and JIA did the analysis, and all authors prepared and approved the final manuscript.

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