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Assessment of Oxidative Stress and Antioxidant Status in Children with Acute Lymphoblastic Leukemia at Diagnosis

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Abstract

Background: Oxidative stress is an inevitable accompaniment in almost every disease process including cancer. As Acute Lymphoblastic Leukemia (ALL) is a disease very much susceptible to oxidative stress, assessment of Plasma Malondialdehyde (MDA) and Erythrocyte Glutathione (GSH) levels are thought of as suitable parameters to detect oxidative stress and antioxidant status respectively in children with ALL. The purpose of the study was to assess the oxidative stress together with antioxidant defense by estimation of plasma MDA and erythrocyte GSH in children with acute lymphoblastic leukemia at diagnosis.

Materials and methods: This prospective case-control study was conducted in the Department of Pediatric Hematology and Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU) in collaboration with the Department of Pharmacology, BSMMU from March 2019 to December 2021. Sixty two (62) children with acute lymphoblastic leukemia were recruited (Group-B) at diagnosis through purposive sampling and 20 control (Healthy children) were enrolled (Group-A). Plasma malondialdehyde and erythrocyte glutathione of these children (Group-A and Group-B) were estimated through spectro photometric measurements and results were compared between two groups of these two parameters by unpaired t-test.

Results: The plasma malondialdehyde levels of Group-B were 3.28±0.40 mol/L (Means ±SD). Plasma malondialdehyde levels of Group-A were 1.17±0.57 mol/L. The difference was statistically significant (p=0.0001). Erythrocyte glutathione levels of group-B were 2.59±1.46mg/g (Means ±SD). On the contrary erythrocyte glutathione levels of group-A were 4.55±1.17mg/g. The difference was statistically significant (p=0004). These results evolve excess MDA production and GSH depletion in children with ALL.

Conclusion: Observation from the study suggested that products of lipid per oxidation (MDA) in children with ALL remained at significantly higher (p=0.0001) at diagnosis. The study also x that malignancy induced stress, body's endogenous antioxidant was being depleted in attempts to detoxify the products of oxidative injury.

Key words : Antioxidant level; Acute lymphoblastic leukemia in children; Oxidative stress.

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Introduction

Cancers are highly stressful diseases among which Acute Lymphoblastic Leukemia (ALL) is a highly renowned disease to impose much oxidative stress upon the patients. ALL is very much well known for its oxidant products and the oxidants produced abundantly in ALL makes the disease a devastating one for both sufferings and survival of the patient. A viscous cycle is created by the oxidative stress induced products which are responsible for mutagenesis of DNA, apoptosis and carcinogenesis; carcinogenesis on the other hand produces products of oxidative reaction or oxidants which would cause further injury to cells and tissues.

Childhood cancer is a very sensitive issue to parents, to the society and therefore to each of the unations of the world. Among all cancers, leukemia's are the most common childhood cancer and consists of 31% of all cancer cases in children less than 15 years of age.[1] Among the leukemia occurring in children, Acute Lymphoblastic Leukemia (ALL) occupies about 75% of the cases whereas Acute Myeloblastic Leukemia (AML) occupies about 20% of all cases. It thus appears

that ALL is the most common childhood cancer in Bangladesh as well as around the world.[2] There is no national population based cancer registry but using worldwide incidence rates an estimated rate of 6000-9000 new cases per year in Bangladesh are being assumed.[3] BSMMU being the predominant center for treatment of childhood cancers, the relative incidence of malignancies seen at BSMMU. Reported cases in BSMMU in the year 2012 was Acute Lymphoblastic Leukemia (ALL) 58% and Non-Hodgkin Lymphoma (NHL) 11% of the total cancer patients admitted in that year.

The basic hostile nature of ALL makes it a disease at the brim of developing resistance towards treatment. Reasons behind this probable resistance to chemotherapy include oxidative stress due to the generation of Reactive Oxygen Species (ROS) and presence of hypo diploid cells. The ROS generated as a result of oxidative stress are produced in cells which use aerobic metabolism for energy production.[4] and may be either beneficial or harmful.

In a study involving pediatric ALL children receiving intensive chemotherapy and solid tumor patients receiving less intensive chemotherapy, it was observed that the children from the ALL group had significantly lower glutathione levels in plasma. They also had a lower antioxidant capacity.[5] The children suffering from solid tumors had a lower antioxidant content while the thiol content did not differ from that of the Control group.[5] These observations are indicative of greater oxidative stress in ALL children. The ALL children therefore had increased ROS in their body and subsequently a decrease of antioxidant status. These suggest that the increased oxidative stress in ALL children might lead to either cell death or greater sensitivity of tumor cells to therapy. Finally they expect better outcome subsequent to treatment than the ALL children.

Glutathione (GSH) is an important intracellular antioxidant that performs a very important role within a cell or tissue to maintain the redox state, drug detoxification and cellular protection from damage by free radicals, peroxides and toxins.[6] High intracellular GSH levels are important contributors to pathologies like cellular transformation and resistance to radiation and antineoplastic treatments in cancer cells. Increased GSH concentrations have been demonstrated in human cancer tissues including breast, brain, colon, pancreas, lungs, head and neck cancer and leukemia.[7] Researchers have observed elevation in serum MDA in ALL patients compared to those in the Controls and they suggest that free radicals such as superoxide and other oxygen metabolites may be generated from lymphocytes due to lipid per oxidation in paediatric ALL patients.[8] MDA being a byproduct of lipid per oxidation, its levels become elevated in a paediatric ALL patients.[1,9] The purpose of the study was to assess the oxidative stress together with antioxidant defense by estimation of plasma MDA and erythrocyte GSH in children with acute lymphoblastic leukemia at diagnosis.

Materials and methods

It was a prospective case control study conducted from March 2019 to January 2021combinedly in the Department of Paediatric Haematology & Oncology and pharmacology, BSMMU, Dhaka, Bangladesh. Clearance from institutional board of BSMMU was taken earlier. After informed consent from parents or legal guardian, children aged between 1-17 years of both sexes who had been diagnosed as acute lymphoblastic leukemia were included in the study. Patients received chemotherapy prior to presentation at BSMMU, impaired renal & hepatic function having serious infection was excluded from the study. Controls were recruited from children attending at Pediatric outpatient department, BSMMU. Purposive sampling technique was applied to recruit the required number at all patient. Following inclusion in the study, particularizes at patients were recorded in data sheet containing age, sex, demographic and clinical information apparently healthy children selected as control (Group-A). Blood collection was done only once to measure the biochemical parameters for the study. Children diagnosed as acute lymphoblastic leukemia was kept experimental group (Group-B) for assessment of oxidative stress plasma MDA levels were measure buy UV-1800 spectrophotometer (ShimadZo) by Thiobarbituric Acid (TBA) reaction method and erythrocyte Glutathione (GSH) level, was done by Ell man's method in the Department of Pharmacology.

Data were processed and analyzed by using Microsoft office Excel. Data were presented as mean #SD in tables and figures as applicable.
Unpaired students t-test was done to compare plasma MDA and erythrocyte GSH levels between control and experiment groups. p value L 0.05, 0.01, 0.001 were taken as levels of significance.

Results

Sixty two (62) Paediatric ALL patients attending the Paediatric Haematology and Oncology Department of BSMMU were recruited in the Experimental group (Group B) and 20 age matched healthy children were enrolled as Controls (Group A) following inclusion and exclusion criteria. The age of Group A boys was 9.66 ± 2.51 years (Mean ± SD) and in girls this was 10.14 ± 3.13 years (Mean ± SD). The height of boys (Group A) was 137.33 ± 13.57 cm (Mean ± SD) and in girls this was 140.42 +18.02 cm (Mean ± SD). The weight of boys and girls (Group A) were 28.33 ± 5.68 kg (Mean ± SD) and 33.28 ± 12.40 kg (mean ± SD) respectively. The BSA of boys and girls (Group A) were 1.00 ± 0.14 (Mean ± SD) and 1.08 ±0.31 m² (Mean ± SD) respectively. The age (Group B) of the boys was 6.40 ± 2.98 years (Mean + SD) and in girls this was 8.45 ± 3.97 years (Mean ± SD). The height (Group B) of boys was 112.34 ± 17.88 cm (Mean ± SD) and in girls this was 114.75 ± 11.58 cm (mean ± SD). The weight (Group B) of boys and girls were 18.40 ± 6.15 kg (Mean ± SD) and 24.52 ± 13.07 kg (Mean ± SD) respectively. The BSA (Group B) of boys and girls were 0.80 ± 0.23 m² (Mean ± SD) and 0.97 ± 0.33 m² (Mean ± SD) respectively.

The plasma MDA concentrations in Control group (Group A) were 1.17 ±0.57µmol/L(Mean ± SD). In the Group B the plasma MDA concentrations were 3.28 ± 0.40 µmol/L (Mean ± SD). There was a statistically significant (p <0.001) increase in the (Mean ± SD) concentrations of plasma MDA in the children of group B (Experimental group, ALL children) compared to those in group A (Control group, age matched children, Table I & Figure 1).

Table I Plasma MDA concentrations

Groups	No. of patients (n)	Plasma MDA conc. (mean ± SD) (µmol/L)	p-value
Control group (Group A)	20	1.17 ± 0.57	0.0001*
Experimental group (Group B)	62	3.28 ± 0.40	

Data were presented as mean ± SD and were analyzed using unpaired 't'-Test

n = Numbers of patients in each grou.

p-value 0.001 (Highly significant).

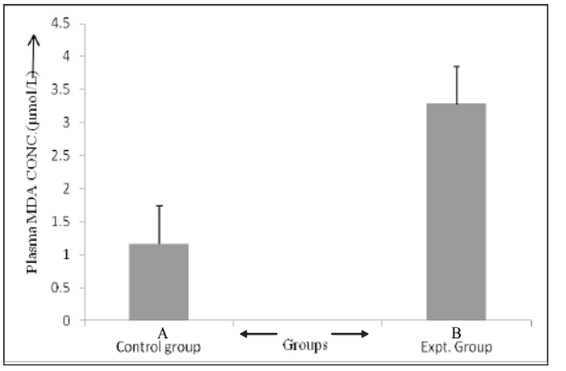

Figure 1 Mean ± SD of plasma MDA concentrations
Group A= Control group.
Group B= Experimental group.

The erythrocyte GSH concentrations in the Control group (Group A) were 4.55 ± 1.17 mg/g (Mean ± SD). In the Group B the erythrocyte GSH concentrations were 2.59 ± 1.46 mg/g (mean ± SD). The reduction in erythrocyte GSH concentrations in ALL children (Group B) was statistically significant (p< 0.001) when compared to those in the Control children (Group A) (Table II & Figure 2).

Table II Erythrocyte GSH concentrations

Groups	No. of patients (n)	Erythrocyte GSH conc. (mg/g of Hb) (mean ± SD)	p-value
Control group (A)	20	4.55 ± 1.17	0.0004*
Experimental group (B)	62	2.59 ± 1.46	

Data were presented as mean ± SD and were analyzed using unpaired 't'-Test.
n = Numbers of patients in each group.
*p -value 0.001 (Highly significant).

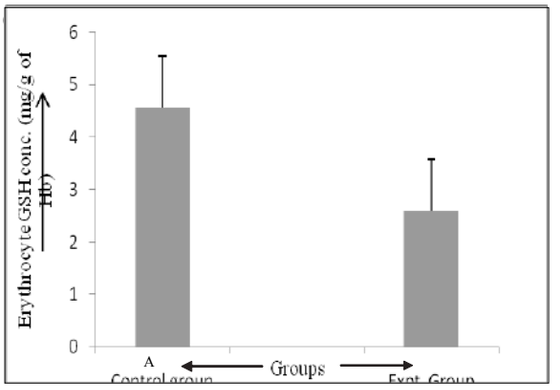

Figure 2 Mean ± SD of erythrocyte GSH concentrations
Group A= Control group.
Group B= Experimental group.

Discussion

Leukemia is the most common childhood cancers representing approximately 31% of all cancer cases occurring in children under the age of 15 years. Acute Lymphoblastic Leukemia (ALL) is a malignant clonal disorder of bone marrow hematopoietic precursor cells. Its onset may be acute or sub-acute. ALL represents approximately 75% of all leukemia within the age group of 15 years.[10] ALL is a devastating cancer, yet intense treatment regimens and the systematic progress in anticancer treatment over the last 40 years has increased the number of childhood cancer survivor's significantly.[11]

Leukemic cells produce higher amounts of Reactive Oxygen Species (ROS) than non-leukemic cells as they undergo repeated phases of oxidative reactions commonly identified as oxidative stress.[12] Oxidative stress is assessed in terms of the Reactive Oxygen Species (ROS) produced and occupies a significant place in carcinogenesis due to resultant DNA damage and its modification. Normal cell types in this way can undergo transformation into malignant cell types. The role of ROS in carcinogenic transformation of normal cells has been studied in different cancer types of adults and in children. Higher amounts of ROS were observed to be produced by cancer cells compared to those by normal cells. Again in detoxifying the ROS, the antioxidant system including antioxidant enzymes of the body was also shown to be reduced or depleted depending upon the severity of the disease process (ALL) and therefore depended upon the amount of ROS production. The antioxidant enzymes in such processes become lower. Quiet good evidence was provided by the fate of antioxidant serum vitamin E which was observed to be reduced in paediatric acute lymphoblastic leukemia.[13]

MDA, which is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid per oxidation was increased at diagnosis in ALL children of the present study. The results indicate a possible link between decreased activities of antioxidant enzymes and increased levels of oxidative damage and support the assumption that free radical reactions may be increased in malignant cells. Plasma Malondialdehyde (MDA) is the principal and highly toxic product of polyunsaturated fatty acid per oxidation, is a marker of oxidative stress occurring in cells or tissues and interacts with DNA to form several different adducts. Normal cellular function is thus inhibited and mutagenesis, carcinogenesis or apoptosis may occur. MDA leads to mutation of DNA and may induce cancer. The amount of this toxic product of lipid per oxidation was estimated in the present research in order to assess the levels of plasma MDA contents which were significantly ($p < 0.001$) increased in the ALL children (Group B) in comparison to those in the Control group (Group B). These results indicate that excess amounts of this toxic product were produced in ALL children in comparison to those produced in the Control children. These results are similar to previous researches which report about increase of serum MDA levels in patients suffering from acute lymphoblastic leukemia.[14] MDA is the main product of lipid per oxidation and is a widely used marker for assessing lipid per oxidation, oxidative stress, DNA damage and cellular damage.[15] Elevation in plasma concentrations of this toxic biomarker in paediatric ALL patients (Group B) of the present study indicates cell damage. Cellular damage in ALL children might have occurred as a result of increased amount of ROS produced in the cancerous conditions in ALL children of the present study. However in Control children (Group A) of the present study, the MDA concentrations were remaining at significantly lower levels compared to the ALL patients (Group B).

Glutathione (GSH) the main cellular thiolic compound has a variety of functions in bio-reduction and detoxification processes. Reduced Glutathione (GSH) which is an important endogenous antioxidant was significantly ($p < 0.001$) reduced in ALL children of the Experimental group (Group B) of the present study. This is no wonder that in a devastating disease like acute lymphoblastic leukemia where significantly large amount of ROS production can occur, this important endogenous antioxidant (GSH) might be reduced in attempts to remove toxic products. Antioxidants are working in the human body in the form of antioxidant defense system against the ROS generation or in attempting detoxification of ROS.[16] Previous researchers in this regard have suggested similar depletion of the antioxidant defenses of the body in attempts to detoxification of oxidant products of cancer cells. The decrease in thiol (GSH) levels may represent a depletion of this antioxidant due probably to detoxification of high concentrations of H2O2 and other peroxides formed by cancer $.) Cells are well known that excessive accumulation of reactive oxygen species (ROS) contributes to antioxidant depletion and resulting cellular dysfunction. In addition, oxidative stress induces lipid per oxidation and protein carbonization by inactivating antioxidant enzymes.

In this study the researcher had obtained levels of Glutathione (GSH) significantly (p0.001) at a higher level in ALL patients at diagnosis. It is probable that decreased GSH levels and increased levels of MDA may occur due to oxidant induced cellular damage supporting the idea that there is persistence of oxidative stress in pediatric acute lymphoblastic leukemia patients of the present study.[17] The decrease in thiol levels may represent a depletion of this antioxidant due to high concentrations of H2O2 and other peroxides formed in tumor cells. It appears that in presence of severe stressful conditions like ALL, thiol levels may reduce to insufficient levels during scavenging ROS during the process of preventing or antagonizing oxidative stress in the affected leukemic cells.

GSH deficiency or reduced GSH levels may be responsible for the immunological non-responsiveness as GSH acts as scavenger of ROS.[18] The present study has observed both increase of the lipid per oxidation marker (MDA) and decreased in the levels of anti-oxidant erythrocyte GSH in the paediatric Acute Lymphoblastic Leukemia (ALL) patients at diagnosis. The present study has obtained observations suggesting low anti-oxidant levels in paediatric ALL patients, which in this regard is similar to previous researches.[19] Free radicals produced in abundance in such cancers may induce oxidative damage to DNA and may produce further development of cancer. The oxidants produced during the cancerous process may further be implicated in chemotherapy-related adverse effects.[20] Cancer patients have been noted to have low anti-oxidant levels at diagnosis.[21] It is therefore recommended that antioxidant supplementation may be advocated along with chemotherapy in paediatric ALL patients for reducing morbidity and mortality. Because oxidants produced during the cancerous condition may damage tissues to produce larger amounts of oxidants or products of lipid per oxidation.

Limitations

This study has several limitations that necessitate mentioning e.g.

- This study did not observe all endogenous oxidants produced in ALL children.
- Total number of patients could not be achieved due to time constraints.
- This was not a multicenter study.

Conclusion

Observations from the present study suggest that the products of lipid per oxidation (MDA) in ALL patients remained at a significantly higher levels compared to those in the Control group. This would indicate that due to ALL induced stress and toxic damage, lipid per oxidation product (MDA) in cancer cells were higher compared to those in the healthy Control children. The results also indicate that due to ALL induced stress and toxic injury produced by the oxidants which were produced in large amounts, body's endogenous antioxidant (GSH) was being depleted to detoxify the products of oxidation. The present study involving paediatric ALL patients suggests that at diagnosis before beginning of treatment, GSH remains reduced or depleted and MDA levels become elevated indicating that a stressful internal milieu prevails.

Recommendation

Assessment of plasma concentration of other endogenous antioxidants in pediatric ALL patients may provide a better assumption of changes in the internal homeostasis of the ALL patients.

- Future research involving administration of antioxidants concurrent to chemotherapy are suggested so that oxidant induced injuries may be prevented.
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Contribution of authors

CSHK-Conception, acquisition of data, drafting & final approval.

ATMAR-Design, critical revision & final approval.

AKMAR-Conception, interpretation of data, critical revision & final approval.

MRI-Data analysis, drafting & final approval.

JM-Acquisition of data, drafting & final approval.

MSI-Interpretation of data, critical revision & final approval.

MH-Data analysis, critical revision & final approval.

FS-Acquisition of data, drafting & final approval.

Disclosure

All the authors declared no competing interests.

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