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Study of Biochemical Markers as Risk Factor for Leukemia in Egyptian Children

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Abstract: The balance of reactive species(ROS) effect is occured by the action of enzymatic antioxidants and non-enzymatic antioxidants. ROS-induced apoptosis is used by the human body to eliminate precancerous and cancerous cells. This study aimed to assess the OS by estimation of malondialdehyde (MDA) , antioxidants as superoxide dismutase (SOD), catalase (CAT), glutathione(GSH) and apoptosis markers as protein (P53) and b-cell lymphoma 2 (BCL2) in patients with ALL of children.This study produced 50 children diagnosed acute lymphoplastic leukemia (ALL) (mean age, 5.8 years) compared to 50 healthy controls (mean age, 5.6 years). Significant decreases obtained in serum P53 (pg/ml) and B-CL2 (U/ml), MDA (nmol/ml) and GSH (nmol/mg) with ALL comparing with the normal control group. But, significant increases were found in serum SOD(U/ml) and CAT(U/L) with ALL compared to the control group ($p<0.001$). .Conclusion: A strong association between apoptosis (P53 and BCL-2) ,oxidative stress(MDA) and (SOD, CAT, GSH) antioxidants with acute lymphoplastic leukemia .

keywords: Oxidative stress(OS), antioxidants, apoptosis.

1.Introduction

Acute lymphoblastic leukemia (ALL) is a type of blood cancers in which large numbers of immature lymphocytes developed.This disease featured by uncontrolled proliferation and lymphoid progenitor cell maturation halt in bone marrow which leads to an excess of malignant cells.Thus normal marrow elements are replaced by lymphoblasts leading to a high decrease in the production of narmal blood cells resulting in varying degrees of anemia, neutropenia and thrombocytopenia. ALL is the most frequent malignancy in children, accounting for more than half of all haematological malignancies in this age group. However, it is uncommon in adulthood, accounting for approximately 2–3% of the population. hematopoietic malignancies. [1]

Like other cancers the causes may be unknown , risk factors may be genetic as Down syndrome, Li-Fraumeni syndrome, or neurufibromatosis type 1.Also it may be due to environmental risk factors like exposure to significant radiation or prior chemotherapy[1].Till now there is no evidence that electromagnetic fields or pesticides are of causes. Some supposed that an abnormal immune response to a common infection may be a trigger. [2,3]

Oxidative stress is related with numerous pathological phenomena like inflammation, infection , ultraviolet and γ-irradiation, increased frequency of mutation and ALL**.** OS is a result of an imbalance between oxygen-free radicals generation or reactive oxygen species (ROS) and antioxidant defense systems response. Free radicals are molecular fragments or molecules which contain one or more unpaired electrons that make them highly reactive. [4]

Free radicals cause lipid peroxidation for the polyunsaturated fatty acids in cell membranes, this may damage the cell structure

and function .Furthermore, deconstruction of lipid peroxidation results in a wide range of end products, including MDA, which is used by many researchers as an indicator of oxidative stress. When everything are normal, circumstances, the body's defense systems, such as antioxidants, play a crucial role in minimizing damage and adapting to stressful situations.[5] The antioxidant action of non-enzymatic antioxidants, as well as antioxidant enzymes, balances the effect of reactive species. Antioxidant defenses have an important in free radicals removal, providing highly protection for biological sites.[6]

In multicellular organisms, apoptosis is a type of planned cell death.[7] Characteristic cell changes (morphology) and death are the result of biochemical events. Blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA degradation are all examples of these alterations. Apoptosis kills between 50 and 70 billion cells per day in adult human.[8]

Approximately 20–30 billion cells die per day for an average human youngster between the ages of 8 and 14.[9] Normal signal transduction pathways and homeostatic systems are disrupted in cancer cells, resulting in anomalies that would induce apoptosis in normal cells. Genomic instability, oncogene activation, and growth factor independent proliferation are examples of these disorders. As a result, cancer cells are likely to require an apoptotic inhibitor in order to survive.[10] Overexpression of the antiapoptotic protein BCL-2 causes apoptosis to be blocked, which is common in cancer cells. Leukemia is thought to be caused by a mismatch between blood cells' ability to proliferate and their ability to die by apoptosis.[11]

The p53 protein has a number of anticancer functions, including apoptosis, genetic stability, and angiogenesis inhibition. P53 has numerous ways that it uses to fight cancer. When DNA is broken, it can activate DNA repair proteins and cause growth arrest by stopping the cell cycle at the G1/S regulation point in response to DNA damage, and trigger apoptosis if DNA damage is irreversible. In unstressed cells, p53 levels are kept low, but they rapidly rise in response DNA damage is one example of a stressor. The P53 gene will thereafter become active through posttranslational modifications and tetramerization following genotoxic or cytotoxic stress .[12,13]

The goal of this research was to figure out: I the oxidative status of ALL patients by measuring lipid peroxidation and levels of advanced oxidation of protein products; and (ii) the oxidative status of ALL patients by measuring lipid peroxidation and levels of advanced oxidation of protein products (MDA) (ii) Antioxidant defense by confirming the key enzymatic antioxidant defenses of SOD and CAT as well as non-enzymatic antioxidant GSH in Egyptian children with ALL and correlation with clinical and hematological results such as lactate dehydrogenase(LDH), hemoglobin (Hb) concentrations and white blood cells(WBCs) count.

2. Materials and methods

The current investigation involved two groups of people: paediatric ALL and healthy children. A total of 50 children with newly diagnosed ALL were involved in the study. Patients were chosen among those who were admitted to the Mansoura Children and Oncology Centers between September 2017 and April 2019, they were 5.8 (3.0-8.0) years old All of the patients or their parents gave their informed consent to take part in the study. All of the patients were diagnosed using established techniques such as cytomorphological, cytochemical, and immunophenotyping. The patient's information was gathered from the patient's archive.

The control group consisted of 50 healthy children with no history of inflammatory diseases in their families. They were 5.6 (3.0-8.0) years old, lived in the same location, and shared the same ethnic background as the patients. They were chosen among youngsters who came to the Mansoura University Children's Hospital's outpatient clinic for a normal checkup and had no history of chronic illness or blood disorders. The study received permission from the local ethical and scientific authorities, as well as informed agreement from all participants in the study.Patient's information included age, sex, domicile, parental consanguinity, family history of

leukemia, occupation, education, and laboratory investigations such as LDH, Hb concentrations, and WBC count.

Samples were collected by taking 5 ml venous blood for assessment of oxidative stress biomarkers, antioxidants and apoptotic markers. MDA level was assayed by Thio-barbituric acid (TBA) test according to the method of Draper and Hadley.[14] SOD was determined by measuring the inhibition of phenazine methosulphate (PMS) according to Nishikimi et al.[15] CAT activity was measured using hydrogen peroxidase as the substrate according to Nishikimi et al previously 's described approach.[15]

GSH level was assayed by GSH is oxidized by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), which produces the yellow derivative 5′-thio-2-nitrobenzoic acid, which is detectable at 412 nm (TNB) according to the method of Rahman et al.[16]BCL2 and P53 levels were measured by Enzyme-Linked Immunosorbent Assay (ELIZA).[17] The samples were allowed to coagulate for 10-15 minutes at room temperature before being collected in a glass centrifuge tube and centrifuged for ten minutes at room temperature (3000rpm).In labeled Eppendorf's tubes, the sera have been frozen at -20oC for special biochemical evaluation.

3. Results and Discussion

As shown in Table 1, There were significant decreases in serum P53 (pg/ml) and B-CL2 (U/ml), MDA (nmol/ml) and GSH (nmol/mg) with ALL in comparison to a healthy control group On the contrary, significant increases in serum SOD(U/ml) and CAT(U/L) with ALL compared to the control group ($p<0.001$) .

Table (1): Biochemical parameters of pediatrics ALL patients and control participants were compared

Parameters	C	ALL	P
N	50	50	
P53 (pg/ml)	0.17±0.02	2.42±0.133	<0.001
BCLl2 (U/ml)	31.8±1.69	31.8±1.69	<0.001
MDA(n mol/ml)	11.9±0.83	27.11±1.81	<0.001
SOD(U/ml)	188.9±13.6	95.3±.1	<0.001
CAT(U/L)	20499±1444	103±9.09	<0.001
GSH(n mol/mg)	0.054±0.062	0.101±0.024	<0.001

C: control; ALL: acute lymphoplastic leukemia;

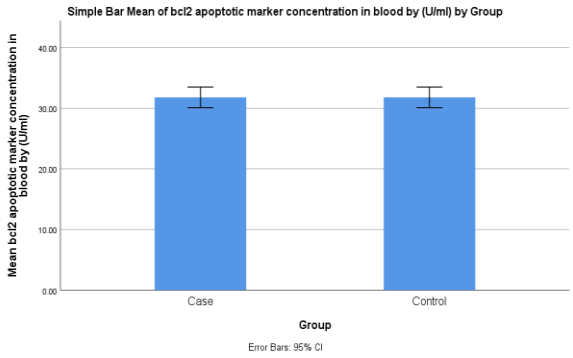
P53: protein; BCL2: b-cell lymphoma 2

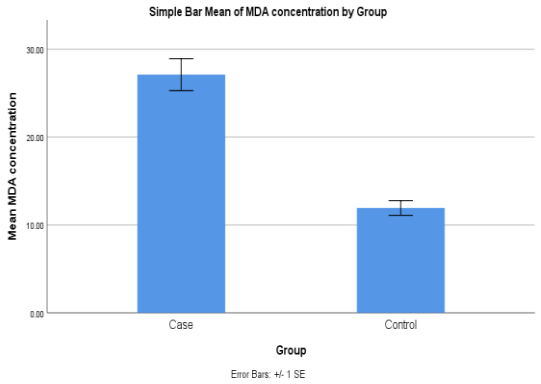
MDA: malondialdehyde; SOD: superoxide dismutase

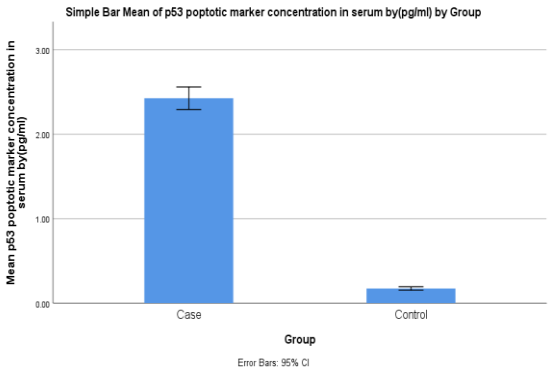
; CAT: catalase; GSH: glutathione.

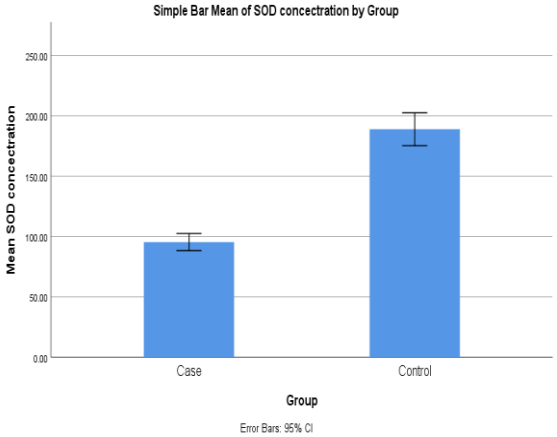
Results are presented as means ±SE for six parameters in each group.

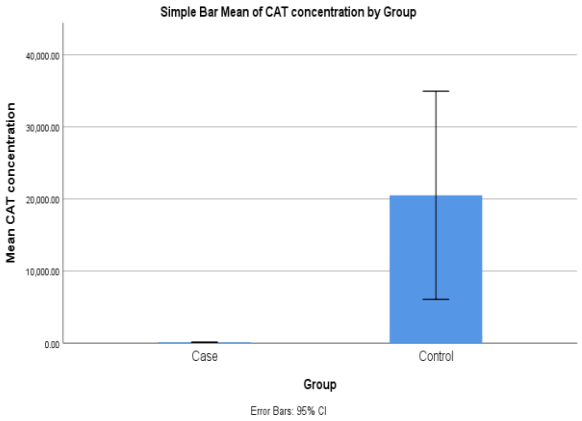
Test of significance is independent samples t-test .











Our findings revealed a substantial positive connection between serum p53, LDH, and WBC count. While Hb concentration has a substantial negative correlation. A strong positive connection was also discovered between serum BCL2 and Hb. WBCs have a substantial negative connection with LDH, but LDH has a significant positive correlation (Table 2).

Table (2): Correlation coefficient between apoptosis parameters and LDH, Hb concentrations and WBCs count in patients.

	P53	Bcl2
WBCs	0.15*	-0.17*
Hb	-0.10*	0.08
LDH	0.29*	-0.20*

Also, there is a statistically significant difference between P53 level and LDH value ($p<0.05$) (Table 3).

Table (3): Significant correlation between apoptosis parameters and the hematological data and LDH in patients.

	P53	Bcl2
WBCs	0.27	0.23
Hb	0.48	0.54
LDH	0.03*	0.15

The current study's findings demonstrate that MDA levels rise when compared to the control/normal group, indicating that oxidative stress is present. A significant hallmark of leukaemia and other malignancies is the impairment of antioxidant enzymes. The results showed that all examined samples exhibited elevated mean concentrations of MDA in control subjects were (11.9±0.83 n mol/ml) comparison to in ALL patients were (27.11±1.81 n mol/ml) these results indicated to 49.5% increasing in MDA concentration present in ALL patients with significant difference at $p< 0.001$.This result was comparable with the previous result obtained by Rasool et al.[18] who discovered that MDA levels rise when compared to control/normal, indicating oxidative stress. A significant hallmark of leukemias and other malignancies is the impairment of antioxidant enzymes. Because the levels of individuals diagnosed with ALL differed significantly from healthy controls, analysis of oxidative stress using MDA as a biomarker proved adequate for acute lymphoblastic leukemias in young patients, indicating a possible direct link between the disease and lipid peroxidation observed in the organism.

Also, Mahmoud et al.19 reported that MDA levels, which have been used as lipid peroxidation indicators, were found to be significantly higher in ALL patients before or during treatment as compared to the control group by (51%) with a significant difference at p0.005. The role of oxidative stress in leukemia relapse in Tunisian patients with acute lymphoblastic leukemia. This finding is consistent with prior research that found a large increase in lipid peroxidation in leukemia patients, possibly as a result of oxidative stress caused by free radical generation, as well as another research. Rajeshwari and colleagues 5 MDA levels in the blood serve as a diagnostic and predictive biomarker for leukemia, suggesting disease progression. Radhakrishnan and colleagues.[2]

Here in, also the activities of SOD, CAT, GSH were assayed as markers of antioxidants status. This study showed that antioxidant defense mechanisms are affected in patients with acute lymphoblastic leukemia disease as indicated by decreased activity of serum SOD and CAT enzymes activities in contrast to an elevation in serum GSH enzyme activity. The findings of this investigation revealed that in all examined samples it was found that SOD mean activity in ALL was (87.4 U/ml) comparing with the control subjects that was (184.5 U/ml),this result indicated that there was 47.3% decreasing in SOD activity with significant difference at $P<0.001$.

Also regarding to CAT the present study showed that ,comparing with control subjects there was significant reduction of CAT activity

recorded in patients with acute lymphoplastic leukemia , where CAT mean activity in control subjects was (563 U/L) , while in ALL patients was (92 U/L).This result indicated 16% decrease in CAT activity in ALL patients with significant difference at P<0.001.

The findings are consistent with those of Sentuerker et al.[21], who found lower CAT and SOD activity in ALL patients' lymphocytes. These data, considered together, show that there are changes in enzymatic antioxidant defenses, which could interfere with the direct removal of free radicals and the protection of biological sites. Also, Battisti V et al.[22]provided results that suggest a reduction in CAT activity in ALL newly diagnosed patients as compared to controls.

Various cancer cells have been revealed to have a cellular redox imbalance caused by oxidative damage. Antioxidant enzymes like CAT and SOD counteract the effects of reactive oxygen and nitrogen species. The loss of CAT and SOD's antioxidant function may aid in the formation of free radicals. Alternatively, it's likely that the antioxidant system is harmed as a result of a cancer-related aberration in antioxidative metabolism.

Other studies, in contrast to the results of this study, have found that SOD and CAT levels in ALL patients increased when compared to the control group Mahmoud et al. 19 In addition, Nishiura et al.[23]discovered that acute leukaemia patients had high serum SOD activity and that leukaemia regression was followed by a decline in SOD levels in the blood.

This could be because, at low levels, SOD protects cells from oxidative stress by catalysing the dismutation of superoxide anion produced in the cell, but at high levels, this enzyme acts as a peroxidase, increasing hydrogen peroxide synthesis and causing cell harm.[24]

In the current study, there was a substantial reduction in GSH activity in patients with acute lymphoplastic leukaemia, with GSH mean activity in control participants (0.04 nmols/mg) and (0.06 nmols/mg) in ALL patients. This findings showed that ALL patients had a 66.6 percent increase in GSH activity, with a significant difference at P< 0.001. The findings back up those of Mahmoud et al.[19], who discovered higher GSH levels in ALL patients as compared to controls. This finding is consistent with Ribeiro et al.[25]'s findings, which demonstrate a link between GSH and ALL patients' relapse and survival. Increased GSH levels have been linked to treatment resistance in tumour cells, according to research. Enhanced GSH levels lead to increased cell survival even when chemotherapeutic medicines are present, resulting in chemotherapeutic drug resistance and disease relapse. Other investigations by Mahmoud et al.[19], in contrast to the findings of this study, imply that the decrease in the activity of this antioxidant enzyme reported in ALL patients can contribute to an increase in H2O2, plasma levels, and other peroxides, and therefore to oxidative stress.

DNA synthesis and repair are both aided by GSH. Reduced GSH levels in leukaemia indicate a depletion of non-enzymatic antioxidant reserves. It reflects the depletion of GSH, which is generally an antioxidant component. GSH shortage, according to Li et al.[26], may be the cause of immunological non-responsiveness to excessive antigenic stimulation by non-professional stimulator cells. Reduced GSH is a crucial critical component that serves as a free radical scavenger.

Cases had much greater levels of Bcl-2 than controls, while resistant cases had significantly higher levels than those who responded to treatment. [27] Srinivas et al.[28] discovered high Bcl-2 levels in primary leukemic cells and a negative connection between apoptotic index and Bcl-2 protein expression in a similar investigation. High levels of BCL-2 expression have been linked to poor treatment outcomes in haematological malignancies such as follicular lymphoma, chronic lymphocytic leukaemia (CLL), and acute myeloid leukaemia (AML) Narayan et al.[29]

In comparison to the control group, we detected a highly significant increase in p53 expression in children with ALL upon diagnosis. This is in line with the findings of Park et al.[30], who used an immunohistochemical approach to show that p53 was overexpressed in both acute

lymphoblastic and acute myeloid leukaemia patients. Mohamed Abdel-Aziz.[31] used the ELISA technique to examine the blood level of p53 in patients with ALL and AML, and discovered a substantial rise in serum p53 in those patients when compared to healthy controls.

Konikova et al.[32] conducted another study that used our technology, flowcytometry, to assess p53, but it was limited to AML cases alone, and it found that AML patients had significantly higher p53 expression than healthy people.

Raida et al.[33] conducted a research that coincides with our findings and used flowcytometry as well. It included children with ALL. It indicated a large increase in p53 expression at diagnosis compared to healthy controls, followed by a considerable drop in complete remission.

At the time of diagnosis, there was a substantial difference between the control and patient groups in terms of LDH, Hb, and WBC count. A substantial positive association was also discovered between serum p53 and LDH, as well as the number of WBCs. While HB and platelets have a considerable negative association. WBCs and LDH have a negative connection as well.

In our patients, we looked at the relationships between serum p53, haematological parameters, and LDH levels. LDH and WBC count showed a strong positive link, while Hb and platelets showed a substantial negative correlation. This is due to p53's anti-apoptotic action, which causes malignant (leukemic) cells to multiply and penetrate B.M. These associations are consistent with Paydas et al.[34]'s findings of a positive connection with LDH and Nakayama and Kamihara[35]'s findings of a positive correlation with WBC count. Furthermore, our findings are consistent with those of Kamal Elden et al.[36], who discovered a positive association between survivin expression and the number of WBCs and blast cells in both peripheral blood and bone marrow. According to our findings, serum p53 has a positive relationship with a number of negative prognostic variables such as high LDH levels and increased WBCs.

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