Glutaredoxin-1 and Others Antioxidants Enzymes in Two Subtypes (B-cell and T-cell) of Acute Lymphoblastic Leukemia Patients

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ABSTRACT

The 5-year event-free viability for acute lymphoblastic leukemia (ALL) have enhanced in many study groups due to modern paediatric clinical trials, however T-cell ALL results are still lagging by 5–10% in most studies. The goal of this study was to measure the Glutaredoxin-1(Grx1) as an antioxidant, and also to measure each of the methionine sulfoxide reductaseA(MsrA),myeloperoxidase(MPO), catalase(CAT), xanthine oxidase(XO), glutathione (GSH), albumin (Alb), and complete blood counts (CBC) in two subtypes (B-cell and T-cell) of ALL patients compared with control group between the ages of two and fifteen years. Revealed with the results of this study, Grx1, CAT, MDA levels increased statistically significantly in two subtypes of acute lymphocytic leukemia Patients compared with control group, but MsrA, GSH, Alb, and CBC levels declined considerably in two subtypes for patients compared with control group. Finally MPO have non-significant difference in compared.The study concluded that there was a high increase in the level of Grx1, which participates in rescuing blood components from the process of breakdown in which a decrease was observed n for two subtypes (B-cell and T-cell), in addition to a decrease in MsrA, GSH, which works to repair the damage occurring in the proteins of cell components, including blood components, malignant B cells are particularly sensitive in contrast to the T cell.

1. Introduction

Acute lymphoblastic leukemia grow in both adults and children, and between one and four years of age it reaches its peak incidence. Predisposing factors have been identified, Such as transmitted genetic template or exposure to the atmosphere. Involvement in the differential and multiplication of lymphoid progenitor cells is characterized by chromosomal abnormalities and genetic alterations, and these disorders are important prognosis parts. Outcomes for patients with acute lymphocytic leukemia are drastically improved by disease risk stratification and the development of treatment protocols. Intensive chemotherapy, particularly in children, but also in teenagers and young adults. Outcomes of relapsed or treatment-resistant patients remain poor in the elderly (≥40 years) and patients who suffer from of lymphoblastic leukemia. New immunotherapeutic strategies are currently being developed, such as antibodies and monoclonal chimeric antigen receptor T cells[1].By the World Health Organization (WHO) they have all been classified into the following subtypes, called “immunotypes” based on a distinct set of proteins present on the cell[2]:

- B-cell lymphocytic leukemia: It constitutes about 75% and 88% of cases among adults and children are chronic lymphocytic leukemia present in the blood and bone marrow.
- T-cell lymphoblastic leukemia: About 25% and 12% of cases in adults and children respectively are of the T-cell lineage of acute lymphoblastic leukemia (ALL).

Figure 1 shows a micrograph of B and T cells and a normal image of a blood stain. Leukemia is primarily and traditionally diagnostic by taking blood smears and examining the bone marrow under a microscope[3]. About 10-15% of all recently diagnostic caseload of pediatric ALL are T-cell ALL (T-ALL). In the past, T-ALL was thought to have a worse prognosis; However, with more advanced treatment, T-ALL and B-cell ALL (B-ALL) now have similar outcomes[4].

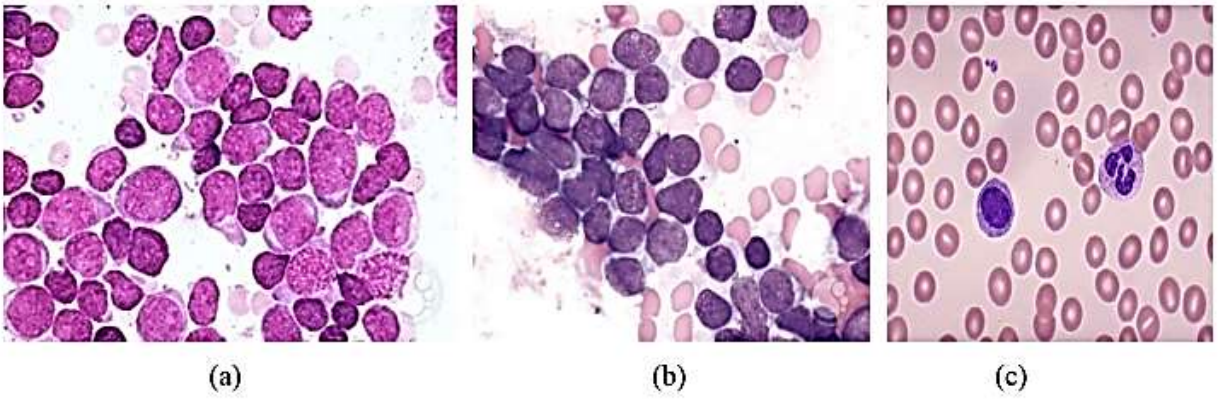

Figure 1: Blood smear images. (a) B-ALL, (b) T-ALL and (c) Normal blood [3].

Bone marrow monitoring plays a critical role in haematology, Size, form, function, and lifetime of the cells during their existence in the bloodstream can be impacted by a number of illnesses, deficiencies, and disorders. They can also cause the bone marrow to leak aberrant or immature cells into the bloodstream, as is definitely the case with anemia and leukemia. White blood cells (WBCs) are produced in excess when leukemia, a blood cancer, develops, generally in the bone marrow. Leukemia affected 2.3 million individuals in 2015 and resulted in 353500 deaths. In 2018, 437033 new cases were detected with the disease. Being more prevalent in the industrialized world, It is the most type of cancer among children, the twelfth common type of cancer, and the eleventh most usual cause of cancer-related deaths casualty [5].In 1976, Arne Holmgren made the discovery of glutaredoxin, or Grx, a tiny protein with an active site cysteine pair. The Grx family has expanded significantly since it was first discovered in *Escherichia coli*, especially in the past 20 years. The system, consisting of Grx, GSH, GRd, and NADPH, initially functions as an electron grantor for ribonucleotide reductase. Different organisms (ranging from bacteria to humans) have been reported to possess multiple isoforms, each serving different functions. Because Grxs may catalyze the combination of GSH to a substrate (Glutathionylation) and the subsequent deglutathionylation, they are unique in the way that they can catalyze glutathione-dependent redox control. Recently, Grxs have also been included in the creation of iron-sulfur clusters. Grx may be a target for medication because these roles have been suggested in a number of bodily and pathologic diseases, including cancer formation, neurodegeneration, and immunological response[6].

MsrA is reduces the oxidation of methionine (Met-O) in biological compounds to methionine (Met), meaning it is an oxidation repair enzyme. Its essential role in cellular processes may be demonstrated by the expression, silencing, or destruction of MsrA or deletion of the gene that Msr A is encoded in many types[7]. Under physiological conditions and oxidative stress (OS) occur in biological system; the common phenomenon is methionine oxidation and reduction. In a cell or organ, methionine sulfoxide (MetO) levels depend on the redox state. It may change its function or cause accumulation of toxic proteins due to molecular modification of proteins. Accordingly, the level of the redox molecules Meto and the associated MsrA system are regulated by the ubiquitous and evolutionarily conserved methionine[8].Aim the article to determination of Grx1 and the levels of antioxidants and other oxidants (MsrA, MPO, CAT, LOX, LP, GST, XO, Alb, GSH, ONOO-, MDA) in B and T cells and their effect on the cell and their evaluation, and also the effect of blood components (Hb, Platelet, PCV, WBC, RBC) on two subtypes of ALL patients, with their evaluation.

2. Materials And Methods

This research included 90 people, 64 ALL patients and 26 healthy people, and the patient samples were separated into two subtype. B cell ALL (B-ALL) and T cell ALL (T-ALL). Samples were collected

from a dedicated pediatric leukemia teaching hospital from Dohuk and Mosul during September to December 2023. The entire medical history of each patient was gathered, and five ml of venous blood was extracted from each participant in this study. The blood was divided into two tubes, one for complete blood test (CBC), and another tube to complete the separation of blood serum was isolated, frozen, and used in tests for biochemical parameters: Grx1 assessed using an ELISA kit from China Biotest Technology Laboratory, GST catalyzes the association of compounds containing electrophilic groups such as 1-chloro-2,4-dinitropenzene (CDNB) [9]. LOX activity was estimated by method of researchers [10],myeloperoxidase activity was calculated by method of researchers [11]. Then, methionine sulfoxide reductase activity was calculated by the method used by the researchers [12], Xanthine oxidase activity was calculated by the procedure used by the researchers [13], Lactoperoxidase an enzyme oxidizes Pyrogallol according to a method [14],and finally, catalase activity in blood serum was estimated based on the standard method. To determine enzyme activity [15], which is based on the oxidation of molybdenum-4-ammonium. As well as a complete blood test, we measured it with a CBC Analyzer Swelab alpha basic made in Sweed.

Statistical Analysis:

The data is tabled as means with standard diversion (SD). A considerable variance was regarded when *P*-values was ≤ 0.05 using t-test [16].

3. Results and Discussion

In latest research, the comparative of the mean values of Grx1 and others antioxidants and oxidants levels in two subtypes (B-cell and T-cell) of ALL patients compared with control group as can be seen **Table (1)**, in Grx1, Cat, LP, GST, ONOO- and MDA showed a meaningful height in subtype B-cell, then subtype T-cell but increased in LOX and XO in subtype T-cell, then subtype B-cell compared with control group. While the results showed a significant decline in the means of MsrA, Alb and GSH in subtype B-cell, then subtype T-cell comparative with control group.

Table 1: Grx1 and others antioxidants and oxidants levels in two subtypes (B-cell and T-cell) of ALL patients compared with control group.

Biochemical parameters	Control		Type B-cell		Type T-cell	
	X	SD	X	SD	X	SD
Age(Year)	8.39 **a**	0.51	9.04 **a**	1.27	8.36 **a**	1.24
BMI (Kg/m^2)	18.14 **a**	1.09	18.47 **a**	8.52	17.95 **a**	0.51
Grx1 (ng/mL)	347.77 **a**	71.83	438.39 **c**	66.86	383.46 **b**	74.59
MsrA (U/L)	513.78 **b**	63.00	436.57 **a**	53.60	412.50 **a**	42.48
Cat (mKat/L)	101.17 **a**	13.25	161.97 **c**	30.41	107.91 **b**	31.47
MPO (U/L)	156.20 **a**	12.63	181.10 **b**	28.92	173.13 **b**	22.98
LOX (U/L)	42.17 **a**	3.37	62.66 **b**	3.64	72.82 **c**	4.31
XO (U/L)	121.20 **a**	76.96	295.32 **b**	74.30	403.96 **c**	71.75
LP (U/ml)	41.93 **a**	3.24	66.55 **c**	6.20	54.84 **b**	5.10
GST (U/L)	219.83 **a**	35.52	428.15 **c**	36.14	285.67 **b**	47.24
Alb (g/L)	43.21 **b**	2.77	30.55 **a**	2.98	32.24 **a**	3.13
GSH (µmol/L)	15.07 **c**	0.90	7.38 **a**	2.66	10.98 **b**	5.34
ONOO- (µmol/L)	72.69 **a**	70.52	103.37 **c**	21.76	96.52 **b**	10.53
MDA (µmol/L)	8.16 **a**	1.74	13.61 **c**	2.61	11.69 **b**	1.08

-Different letters (a, b, c) in horizontal indicate that the means are different significantly at p≤0.05, among the studied groups.

The results showed a considerable decrease in the mean values of Hb, RBC, Platelet, PCV% in subtype for ALL patients compared with control group; as can be seen **Table (2)**, a significant decline in WBC subtype B-cell then T-cell when compared with control group.

Table 2: Levels of CBC in two subtypes of ALL patients compared with control group .

Blood components	control		Type B-cell		Type T-cell	
	X	SD	X	SD	X	SD
Hb (g/dL)	13.11 **b**	0.30	10.46 **a**	0.95	10.00 **a**	0.59
RBC (10^6/uL)	4.63 **b**	0.076	3.50 **a**	0.32	3.41 **a**	0.131
WBC (10^3/uL)	6.30 **c**	0.26	3.39 **a**	0.76	4.03 **b**	0.75
Platelet(10^3/uL)	251.77 **b**	11.10	206.68 **a**	29.22	202.12 **a**	57.22
PCV%	33.68 **b**	0.73	30.71 **a**	3.22	28.51 **a**	3.11

Oxidative stress (OS) has a double role in the fate of malignant tumors. In B-cell malignancies, redox disequilibrium depends on concentration, and localization of ROS. What makes malignant cells resistant to treatment is that malignant cells adapt to reduce their virulence and increase their energy requirements by increasing their antioxidant capability and their effector immune function may be negatively affected by the microenvironment and OS [17,18].In a latest study, the level of GRX1 in B cells was found to be higher than in T cells. This is because in growth and response pathways compared to T cells that depend critically on Trx, B cells are more elastic. They usually use both pathways (TrxR1 and GSH/Grx1). When one is not sufficient for B cells to ensure redox remodeling, because B cells are exhibited to higher levels of reactive oxygen species (ROS) than T cells, which may explain, from an evolutionary perspective, the need for a more strong antioxidant system to maintain redox homeostasis in the cytosol. In granulocytes and macrophages, NADPH oxidase (NOX), which is expressed and used to kill microbes, is also an important contributor to ROS in B cells but not T cells. B cells express NOX1 and double oxidase 1 (DUOX1) among the enzymes. NOx varies and this is consistent with scientists' research [19].In a latest study, the level of MsrA decreased considerably,but the same effect of T and B cells, and as we noticed that the level of MSRA decreased, and this is consistent with the research of researcher Moskovitz and Smith [8], usually under conditions of OS, aging, inflammation, and diseases associated with OS, it does not increase MSR level.The important methods of GSH and Trx, and other antioxidants such as superoxide dismutase and catalase are preserved as endogenous antioxidants. Methionine sulfoxide reductases (MSRs) comprise a unique group. It is an antioxidant that can reduce methionine sulfoxide in proteins, as a general cellular antioxidant that also scavenge free radicals [20]. In healthy cells, ROS are often created in cells and tissues as a byproduct of metabolism. Through apoptosis they can be generated further. External mechanisms and factors such as chemotherapy drugs methionine S and R are reduced in sulfoxides proteins. By the separate MsrA and MsrB, severally, free methionine-S-sulfoxide is also reduced by MsrA. It contributes to free radical scanning and may enhance the accessibility of reduced form methionine as a methyl donor for epigenetic DNA methylation. MsrA has nuclear/cytoplasmic and mitochondrial isoforms produced from the MSRA gene in humans. They contain cysteine residues that are essential for redox activity. It enters the protein through the substrate and forms a cysteine disulfide bond. By thioredoxin system MsrA is reactivated and there is also testimony of activation by glutaredoxins [21].We also noticed in a recent study that the level of MDA is higher in B cells than in T cells, and the reason is as explained by scientists [22]. They said that the excessive production of ROS, which mediates the functional capacity of cellular antioxidants, may destabilize important molecules. It represents the molecular basis of many diseases including inflammatory processes, cardiovascular changes, cancer, etc. An increase in lipid peroxidation and an increase in MDA have been observed in patients or children with acute leukemia. Under normal physiological conditions, MDA is rapidly oxidized to acetate or malonate, and then in the Krebs cycle. Excess MDA interacts with various serum proteins and cell membrane components to form altered determinants. One possibility is that it could also interact with DNA and inhibit the biosynthesis of DNA, RNA, and proteins. Its tumorigenic potential has been shown to be postulated because it contains the structure of various carcinogenic MDA compounds such as glyceraldehyde and beta-propiolactone. It can therefore be hypothesized in this study that the significantly higher level of MDA detected in our children with ALL could exacerbate DNA damage

and carcinogenesis. Included. In another study, the increase in MDA detected in ALL was demonstrated [23].In a recent study, it was shown that the levels of Grx and CAT enzymes increased significantly. This is one of the reasons, given by the researchers [17]. In B cells, one of the elements that contribute to the aetiology and development of malignant tumors is an imbalance in redox metabolism. There is also a strong association between high levels of OS and the advanced primitive stage of various B-cell malignancies. The effect of ROS is not limited to malignant cells, but also affects neighboring cells as well as cancer cells, including immune cells, in the complex present in the tumor microenvironment. It has the ability to adapt to increasing energy demand and reduce its offsets. This may cause malfunctioning, leading to immune avoidance and tumor development .This makes malignant B cells particularly vulnerable to increased oxidants compounds due to unbalanced redox balance. It has already shown anti-leukemia activity in preclinical models and has been targeted for antioxidant systems which means that there is a high increase in (Grx1, CAT, MDA) and a decrease in antioxidants of (GSH, Alb) and CBC.GSH is reduced through its function as a controller of the cellular redox state, protecting cells from hurt caused by oxidant compounds, lipid peroxides, and antibiotics as an important intracellular antioxidant. In healthy cells, it is critical for the removal and detoxification of carcinogens, and high GSH levels in cancer cells are associated with tumor advancement and increased resistor to chemotherapy drugs [24]. Also, this study showed us that the percentage of GSH is higher in the T cell than in the B cell, and the reason is that inside the cells, a lack of GSH leads to an increase in ROS. It disrupts redox balance, touching cell function and survival. Because of the difference Degrees of OS in Tregs and T17 cells, GSH Deficiency can both promote and inhibit Treg distinction T17 distinction, resulting in an imbalance between Treg and T17 differentiation [25].

The level of MPO increases in cancer cells and the reason for this is. It has the ability to generate many reactive species, essential for host fungal defense and antimicrobial activity. However, tissue damage can occur due to unregulated MPO release, as seen in many diseases. Thus, increased circulating MPO levels are widely associated with conditions of increased inflammation, OS, and inflammation [26]. The carcinogenic environment is enhanced by the enzymatic activity of MPO, and the resulting oxidants promote several axes of carcinogenesis [27]. MPO activity is a double-edged sword that boosts innate immunity in the process of removed cancer cells, but at the same time administers mutagenic potential and disturbs the extracellular space, allowing tumors to infiltrate their surroundings and diffusion more rapidly. [28].CAT in two subtypes of ALL increased. The reason is that CAT reacts with hydrogen peroxide generated by SOD, or from other substances, and decomposes it into molecular oxygen and water [29]. By removing toxins, the antioxidant CAT plays a key role. However, there is a shuttle between this organelle and the cytoplasm and may have a role in protecting key cellular elements (e.g. proteins,…chromosomes) against oxidative damage. It has also been shown in cancer cells that catalase expression is altered, likely leading to cell proliferation by activating oncogenes and causing genomic instability. Catalase regulation and expression are primarily controlled at transcriptional levels, but other mechanisms are also possible. Therefore, utilization of pro-oxidant catalase pathways has the potential to be a future therapeutic target in the context of cancer [30]. As we explained before, CAT is an antioxidant that increases more in the B cell than in the T cell Because B cells are exhibited to higher levels of ROS than T cells.Lipooxygenase increased in this study due to are LOXs enzymes that catalyze the oxidation of polyunsaturated fatty acids that can form lipid metabolites implicated in many biological roles, including OS [31]. As we have noticed, the effect of the LOX on B cells and T cells increases in the T cell more than in the B cell due to the effect of fats on two of these cells. For example, defects in the arachidonate 5-lipoxygenase ALOX5 also affect the generation of memory cells B, which would fully support the maintenance of the major B cell repertoire possessed by memory B cells and naïve cells. It is mainly related with a variety of inflammatory diseases, for example cancer, asthma, atherosclerosis, cirrhosis, and rheumatoid arthritis, which are caused by inherent abnormalities in acquired immunity [32].In this study, we observed a low level of albumin in a cancer patient in B and T cells. This is due to OS, generation of ROS, and acidosis. This leads to a decrease in its composition or changes in functional activity, causing a violation of fatty

acids, minerals, drugs and hormone transport. And the properties of albumin. Changes under these attacks [33].We observed a significant increase in XO in All patients, and this is due to OS in the body. It is formed as a result of an imbalance between free radicals and antioxidants and is regarded one of the major causes of cell damage and disease growth. Hyperuricemia, the end product of purine metabolism in humans, is carefully linked to the generation of ROS [34].In this study, GST levels are higher in cancer patients compared to healthy people because of this Glutathione (GSH) and glutathione-S-transferases(GSTs) serve as front lines of cellular defensive against both chronic and acute toxicity of xenobiotic- resulted OS [35]. It is known that cancer patients who take medications and chemotherapy cause an increase in this enzyme, and it is higher in B cells than in T cells because B cells are susceptible to higher levels of ROS than T cells.In this research, LP levels were increased in a ALL patients, because from a biological point of view, strongly oxidized H_2O_2 is important. In tissues, it may lead to infection if it is not controlled. Therefore, LP catalyzes a redox reaction even as oxidizing thiocyanate (SCN^-) to the comparatively harmless hypothiocyanate ($OSCN^-$) in order to reduce high levels of H_2O_2 to H_2O. SCN^- is the only natural and active substrate for LP [36].As we know, these free radicals will have harmful effects on all biomolecules. We call it OS, which occurs when the balance between oxidants and antioxidants is disturbed. Superoxide can generate other molecules, and the Various molecules can be produced by oxidation of hydroxyl radical located near it into DNA, phospholipids, and proteins. Free radicals bind to nitric oxide (NO) to afford ONOO -a powerful oxidizing agent without loss. It can cross cell membranes by converting H_2O_2 to $^{\bullet}OH$, which is a strong oxidizing agent such as ozonation of lipid chains and can generate free radical chains that react with a huge number of inorganic substances and compounds of organic [37]. Due to the many harmful effects of free radicals, they will affect cell membranes through the occurrence of lipid peroxidation and oxidation, protein denaturation and DNA damage. All of these effects cause inflammation, immune responses, tumor risks, mutations, and apoptosis [38].A comprehensive blood test is one way to diagnose leukemia. Acute lymphocytic leukemia (ALL) The initial clinical diagnosis is based on the patient's signs and symptoms, peripheral examination, and CBC testing. Bone marrow analysis and blood smear. People with acute lymphoblastic leukemia (ALL) have low numbers of RBC and platelets and high numbers of white blood cells [2]. The majority of cancer patients suffer from anemia. According to a recent study, Hb. level decreases. This is because anemia is common among cancer patients, and with chemotherapy the incidence of it increases. With chemotherapy, the likelihood of needing a blood transfusion also increases. Anemia has a negative effect. Survival in cancer patients and increases fatigue. Cancer stimulates the production of inflammatory cytokines, which inhibit erythropoiesis and erythropoietin production [39].

Conclusions

Finally, it was observed in our study that the levels of Grx1, MsrA, and most of the antioxidants needed by the body rise in B cells, and due to increased oxidant compounds and unbalanced redox homeostasis, B cancer cells are particularly touchy, in contrast to T cells.

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