MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Assessment of some Biochemical Markers as risk factors in Egyptian children affected with Acute Lymphoblastic Leukemia

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Received:11/10/2023
Accepted: 19/11/2023

Abstract: Acute lymphoblastic leukemia (acute lymphocytic leukemia, ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow. The oncogenes that control the production of ROS and the expression of antioxidants have the ability to influence the apoptosis pathway and control the progression of leukemia. This research investigated the inter-relationship between antioxidant markers, oxidative stress, and some biochemical markers in acute lymphoblastic leukemia Egyptian children. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). In addition to oxidative stress markers such as malondialdehyde (MDA). Furthermore, electrolytes such as (Na, K, Cl, Ca, Mg, and P). Moreover, liver profile, kidney profile and Glucose in acute lymphoblastic leukemia Egyptian population. This study produced 100 children diagnosed with acute lymphoblastic leukemia (ALL) (mean age, 9.5 years) compared to 100 healthy controls (mean age, 8.5 years). Significant increases were obtained in Na and K, MDA, and GSH levels in ALL groups compared to the control group. While, we found a significant decrease was found in serum SOD and CAT in ALL groups compared to the control group ($p<0.001$). Conclusion: There is a strong association between electrolytes (Na and K), antioxidants (SOD, CAT, and GSH), and oxidative stress (MDA) with acute lymphoblastic leukemia. Which means, these parameters could be risk factors in ALL.

keywords: ALL, Biochemical markers, Electrolytes, Oxidative stress, Antioxidants.

1.Introduction

Acute lymphocytic leukemia (ALL) is a type of blood cancer that is more prevalent in children. [1] It accounts for almost 30% of pediatric cancers. Despite the high rate of cure, ALL is one of the leading causes of death in children with tumor. For this reason, there is a keen interest in identifying genetic and biological features that influence the pathogenesis of ALL and the risk of treatment failure. [2]

ALL is a hematopoietic neoplasm characterized by the exacerbated proliferation of blasts in bone marrow and affects mainly children aged 2 to 15 years old. [3] The age-adjusted incidence rate of ALL in the United States is 1.38 per 100,000 individuals per year, with approximately 5,930 new cases and 1,500 deaths estimated in 2019**.** [4]

It is also the most common type of cancer in children**,** [5] representing 75%–80% of acute leukemias among children. The median age at diagnosis for ALL is 15 years, with 55.4% of patients diagnosed at younger than 20 years of age. In contrast, 28% of patients are diagnosed at 45 years or older and only approximately 12.3% of patients are diagnosed at 65 years or older**.** [6]

2. Materials and methods

The current study was designed as a case control study, involved two groups of pediatrics: A total of 100 patients with acute lymphoblastic leukemia (64 males and 36 females) ranging from 2.0 – 17.0 years old (Median = 9.5) were collected from the Pediatric department at Oncology center - Mansoura University and a similar number of normal children, age and sex matched, living in the same geographical area (used as a control group) From March 2021 to March 2022. Informed consents were obtained from the parents of children with ALL. The approval of the study was obtained from Mansoura Faculty of Medicine (Research Ethics Committee (REC) and the Institutional Research Board (IRB) MS.20.11.1288). Collected data will not be used for other purpose.

The entire group of patients were diagnosed by formed techniques such as cytomorphological, immunophenotyping and cytochemical. Patients had a full medical history as well as general and local clinical examination with concentrating on the liver, spleen and lymph nodes. The clinical data of patients was collected from the patient's archive.

A total of 100 healthy group (40 males and 60 females) with no history of family with leukemia or any other malignancies. They were the same age and sex, living in the same geographical area (used as a control group).

5 mL of venous blood sample was collected. 3 ml to estimate oxidative stress biomarkers such as the activity of serum malondialdehyde (MDA) level, as well as antioxidants such as catalase (CAT) and superoxide dismutase (SOD). 2 ml to estimate biochemical markers as (Bilirubin, Albumin, ALT, AST, ALP, Total protein, Urea, Creatine, Uric acid and Glucose) and electrolytes as (Na, K, Cl, Ca, Mg and P).

MDA level was assayed by Thio barbituric acid (TBA) test according to the method of [7] SOD was determined by measuring the inhibition of phenazine methosulphate (PMS) according to [8] CAT activity was measured using hydrogen peroxidase as the substrate according to [9] GSH level was assayed by oxidizing GSH using the sulfhydryl reagent 5,5 di thiobis (2-nitrobenzoic acid) (DTNB), which produce the yellow derivative 5-thio-2-nitrobenzoic acid which is detected at 412 nm (TNB) according to the method of**.** [10] Measuring the Concentration of Alkaline phosphatase using Colorimetric method kinetic (Coromatest, Linear Chemicals. S.L) according to the method of [11] Measuring the Concentration of Aspartate aminotransferase AST and Alanine aminotransferase ALT by using Colorimetric method endpoint (Coromatest, Linear Chemicals. S.L) according to the method of [12] Measuring the Concentration of Albumin by using colorimetric method endpoint (Coromatest, Linear Chemicals. S.L) according to the method of [13] Measuring the Concentration of Total Bilirubin by Colorimetric method, end point(Coromatest, Linear Chemicals. S.L) according to the method of [14] Measuring the Concentration of Uric Acid by using Enzymatic colorimetric method endpoint(Coromatest, Linear Chemicals. S.L) according to the method of [15] Measuring the Concentration of Creatinine by using Kinetic colorimetric method (fixed time)(Coromatest, Linear Chemicals. S.L) according to the method of [16] Measuring of Lactate dehydrogenase (LDH) by using UV enzymatic method kinetic(Coromatest, Linear Chemicals. S.L) according to the method of [17] Measuring the concentration of Total protein by using colorimetric method Endpoint(Coromatest, Linear Chemicals. S.L) according to the method of [18] Measuring the concentration of Glucose by using Enzymatic colorimetric method Endpoint(Coromatest, Linear Chemicals. S.L) according to the method of [19] Measuring the concentration of Sodium (Na^+) using Enzymatic method Fixed Time (Coromatest, Linear Chemicals. S.L) according to the method of [20] Measuring the concentration of Potassium (K^+) using Enzymatic method Fixed Time (Coromatest, Linear Chemicals. S.L) according to the method of [21] Measuring the concentration of Calcium (Ca^+) using Colorimetric method endpoint (Coromatest, Linear Chemicals. S.L) according to the method of [22] Measuring the concentration of Magnesium (Mg^+) using Colorimetric method endpoint (Coromatest, Linear Chemicals. S.L) according to the method of.[23] Measuring the

amount of Phosphorus (P^+) using Colorimetric method endpoint (Coromatest, Linear Chemicals. S.L) according to the method of [24] Measuring the concentration of Chloride (Cl^-) by using Colorimetric method endpoint (Coromatest, Linear Chemicals. S.L) according to the method of.[25]

Statistical Analysis

The collected data was revised, coded, tabulated using Statistical package for Social Science **(IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).**

Descriptive statistics:

- Mean, Standard deviation, standard error, median, minimum and maximum for numerical data.
- Frequency and %age of non-numerical data.

Analytical statistics:

- **Student T** Test was used to assess the statistical significance of the difference between two study group means.
- **One Way ANOVA** test was used to assess the statistical significance of the difference between more than two study group parametric variables.
- **Mann Whitney Test (U test)** was used to assess the statistical significance of the difference of a non-parametric variable between two study groups.
- **The Kruskal-Wallis** test is was used to assess the statistical significance of the difference between more than two study group non parametric variables.
- **Chi-Square test** was used to examine the relationship between two qualitative variables. **Monte Carlo test:** was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.
- **Correlation analysis:** To assess the strength of association between two quantitative variables.
- The ROC Curve (**receiver operating characteristic)** provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. The optimum cut off point was defined as that which maximized the AUC value.
- AUC is that a test with an area greater than 0.9 has high accuracy, while 0.7–0.9 indicates moderate accuracy, 0.5–0.7, low accuracy and 0.5 a chance result**.**

Regression analysis: Logistic analysis was used for prediction of risk factors when dependent variable is categorical, using generalized linear models.

An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.

OR=1 Exposure does not affect odds of outcome

OR>1 Exposure associated with higher odds (risk) of outcome.

OR<1 Exposure associated with lower odds of outcome (protective).

The 95 % confidence interval (CI) is used to estimate the precision of the OR. A large CI indicates a low level of precision of the OR, whereas a small CI indicates a higher precision of the OR.

Probability of results

- A *p* value is considered significant if <0.05 at confidence interval 95%.

3. Results and Discussion

Mean (±SD) Bilirubin was 0.56 ± 0.10 (mg/dL), ranged from 0.38 to 0.90. Mean (±SE) ALT was 22.72 ± 0.90 (U/L), ranged from 10.55 to 40.42. Mean (±SD) AST was 24.86 ± 6.78 (U/L), ranged from 16.7 to 38.3. Mean (±SD) ALP was 0.56 ± 0.10 (U/L), ranged from 82.20 to 7.80. Mean (±SE) LDH was 444.20 ± 16.82 (U/L), ranged from 210 to 985. Mean (±SD) Albumin was 4.19 ± 0.44 (mg/dL), ranged from 3.4 to 5.5. Mean (±SD) Total protein was 4.80 ± 0.77 (g/dL), ranged from 2.86 to 5.80. Mean (±SD) Creatine was 0.61 ± 0.17 (mg/dL), ranged from 0.40to 1.20. Mean (±SE) Uric acid was 4.29 ± 0.15 (mg/dL), ranged from 1.55 to 8.10. Mean (±SD) Glucose was 105.62 ± 19.30 (mg/dL), ranged from 70.0 to 138.0.

Table 1. Biochemical markers among patients with ALL.

	ALLn = 100
Bilirubin (mg/dL)	
Mean ± SD.	0.56 ± 0.10
Median (Range)	0.55 (0.38 – 0.90)
ALT (U/L)	
Mean ± SE.	22.72 ± 0.90
Median (Range)	18.32 (10.55 – 40.42)
AST (U/L)	
Mean ± SD.	24.86 ± 6.78
Median (Range)	25.0 (16.7 – 38.3)
ALP (U/L)	
Mean ± SD.	82.20 ± 7.80
Median (Range)	82.94 (66.64 – 97.77)
LDH (U/L)	
Mean ± SE.	444.20 ± 16.82
Median (Range)	460.5 (210.0 – 985.0)
Albumin (g/dL)	
Mean ± SD.	4.19 ± 0.44
Median (Range)	4.2 (3.4 – 5.5)
Total protein (g/dL)	
Mean ± SD.	4.80 ± 0.77
Median (Range)	5.10 (2.86 – 5.80)
Creatine (mg/dL)	
Mean ± SD.	0.61 ± 0.17
Median (Range)	0.60 (0.40 – 1.20)
Uric acid (mg/dL)	
Mean ± SE.	4.29 ± 0.15
Median (Range)	4.46 (1.55 – 8.10)
Glucose (mg/dL)	
Mean ± SD.	105.62 ± 19.30
Median (Range)	106.0 (70.0 – 138.0)

SD, standard deviation, SE, standard error; min, minimum; max, maximum.

Table 2. Electrolytes and trace elements among patients with ALL.

	ALLn = 100
Ca (mg/dL)	
Mean ± SD.	6.86 ± 0.42
Median (Range)	6.8 (5.7 – 7.7)
P (mg/dL)	
Mean ± SD.	5.99 ± 0.36
Median (Range)	6.0 (5.2 – 6.8)
Mg (mg/dL)	
Mean ± SD.	1.97 ± 0.17
Median (Range)	2.0 (1.7 – 2.2)
Na (mmol/L)	
Mean ± SD.	158.43 ± 11.41
Median (Range)	156.1 (134.0 – 192.4)
K (mmol/L)	
Mean ± SD.	6.64 ± 0.48
Median (Range)	6.7 (5.3 – 7.7)
Cl (mmol/L)	
Mean ± SD.	104.93 ± 5.84
Median (Range)	105.5 (95.8 – 114.7)

SD, standard deviation, min, minimum; max, maximum.

Electrolytes and trace elements among patients with ALL are shown in table (2). Mean (±SD) calcium was 6.86 ± 0.42 (mg/dL), ranged from 5.7 to 7.7. Mean (±SD) P was 5.99 ± 0.36 (mg/dL), ranged from 5.2 to 6.8. Mean (±SD) Mg was 1.97 ± 0.17 (mg/dL), ranged from 1.7 to 2.2. Mean (±SD) Na was 158.43 ± 11.41 (mmol/L), ranged from 134 to 192.4. Mean (±SD) K was 6.64 ± 0.48 (mmol/L), ranged from 5.3 to 7.7. Mean (±SD) Cl was 104.93 ± 5.848 (mmol/L), ranged from 95 to 114.7.

MDA, CAT, SOD, GSH among studied groups

Table 3. Comparison of oxidative stress and antioxidants markers among ALL patients and control group.

	ALLn = 100	***Control n = 100***	***Test (p)***
MDA (nmol/mL)			
Mean ± SE.	*1.75 ± 0.16*	*0.49 ± 0.02*	*U =2505.5 p<0.001*
Median	*0.90*	*0.48*	
Range	*0.2 – 6.2*	*0.0 – 0.9*	
GSH (mg/dL)			
Mean ± SE.	*1.74 ± 0.04*	*0.10 ± 0.02*	*U =12.0 p<0.001*
Median	*1.80*	*0.06*	
Range	*0.75 – 2.40*	*0.01 – 1.08*	
SOD (U/L)			
Mean ± SE.	*86.45 ± 4.88*	*242.50 ±7.16*	*U =9549.0 p<0.001*
Median	*86.60*	*249.50*	
Range	*11.47 – 212.50*	*28.50 –367.0*	
CAT (U/L)			
Mean ± SE.	*171.16 ±12.51*	*499.39 ±26.02*	*U =8326.0 p<0.001*
Median	*135.0*	*547.15*	
Range	*25.0 – 731.0*	*26.60 968.75*	

SE, standard error; min, minimum; max, maximum. U, Mann Whitney test.

ALL showed significantly higher MDA (median=0.9 versus 0.5, p<0.001), GSH (median=1.8 versus 0.0.6, p<0.001), significantly lower CAT (median=135 versus 547, p<0.001), and SOD (median=86.6 versus 249.5, p<0.001), when compared to control group.

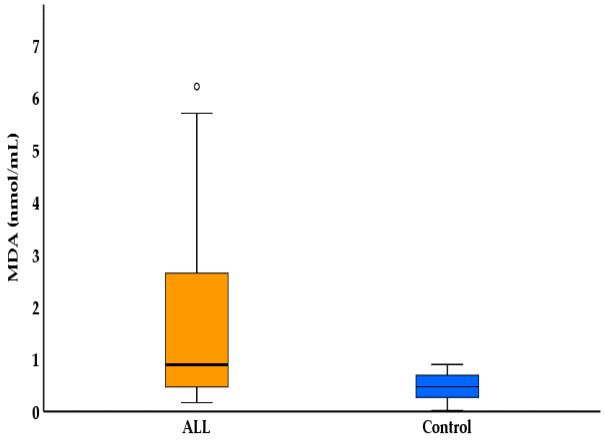

Figure 1. Boxplot for MDA among ALL patients and control group.

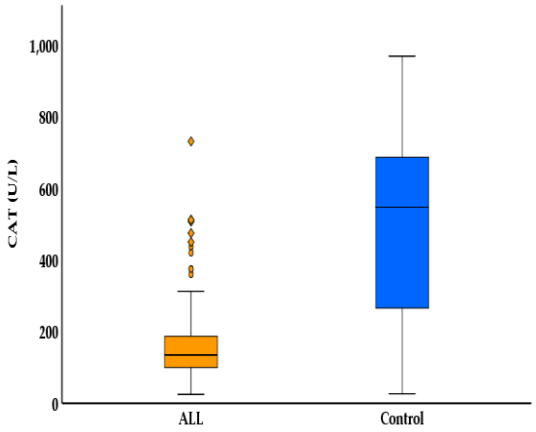

Figure 2. Boxplot for CAT among ALL patients and control group.

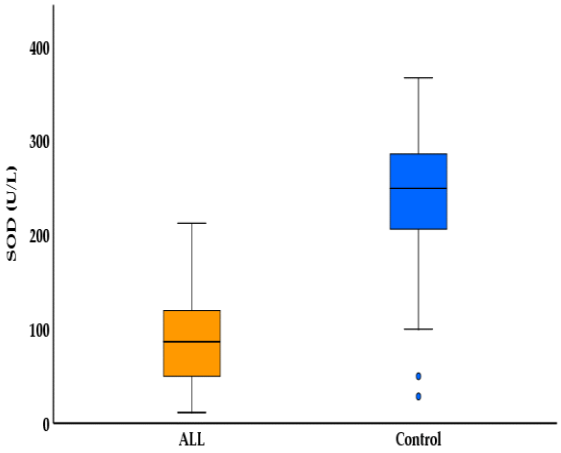

Figure 3. Boxplot for SOD among ALL patients and control group.

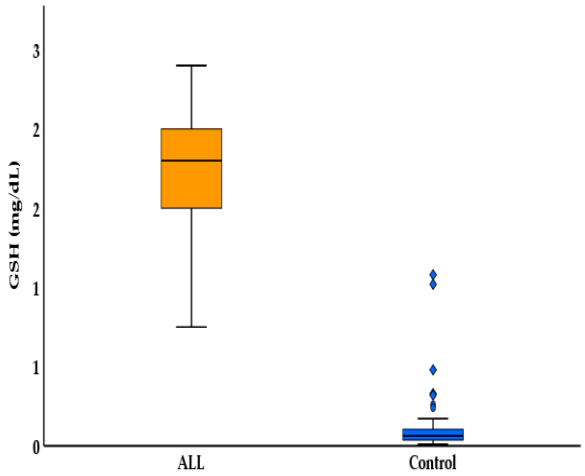

Figure 4. Boxplot for GSH among ALL patients and control group**.**

Table 4 Comparison of Biochemical markers among ALL patients and control group.

	Disease group (n= 100)	**Control group (n= 100)**	**95% CI**	**P**
Bilirubin (mg/dL)	0.56 ± 0.097	0.56 ± 0.102	0.03, 0.02	0.793
Albumin (gm/dl)	4.19 ± 0.441	4.12 ± 0.431	-0.05, 0.20	0.224
ALT (U/L)	22.72 ± 9.001	22.55 ± 8.687	2.30, 2.64	0.893
ALP (U/L)	82.20 ± 7.800	82.95 ± 9.290	3.15, 1.64	0.535
AST (U/L)	36.72 ± 10.756	36.73 ± 12.569	3.28, 3.25	0.992
Total protein (gm/dl)	4.80 ± 0.770	4.77 ± 0.844	0.19, 0.26	0.756
Creatine (mg/dL)	0.60 ± 0.165	0.60 ± 0.149	0.04, 0.05	0.722
Uric acid (mg/dL)	4.29 ± 1.539	4.26 ± 1.605	0.41, 0.47	0.898
Glucose	105.62 ± 19.304	103.03 ± 19.158	2.77, 7.95	0.342
Positive CRP	39 (39.0%	0 (0.0%	-	**< 0.001**
Na (mmol/L)	158.43 ± 11.405	139.55 ± 2.792	16.57, 21.20	**< 0.001**
K (mmol/L)	6.64 ± 0.476	4.58 ± 0.597	1.91, 2.21	**< 0.001**
Cl	104.93 ± 5.839	104.73 ± 5.871	1.43, 1.83	0.811
Mg	1.97 ± 0.174	1.94 ± 0.171	0.02, 0.07	0.287
Ca	6.86 ± 0.416	6.90 ± 0.525	0.18, 0.09	0.499
P	5.99 ± 0.356	5.95 ± 0.478	0.08, 0.15	0.550
Data is expressed as mean and standard deviation or as percentage and frequency. 95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.				

Table 5. Regression analysis for prediction of ALL susceptibility.

	Univariable			Multivariable		
	p	**OR**	**95% CI**	**p**	**OR**	**95% CI**
Age	0.167	0.969	0.9261.013			
Gender	0.883	0.973	0.677-1.399			
MDA	*<0.001*	3.296	2.032-5.347	0.814	1.002	0.987-1.017
CAT	*<0.001*	0.996	0.995-0.997	0.225	0.991	0.987-1.012
SOD	*<0.001*	0.981	0.976-0.985	0.001	0.994	0.991-0.998
GSH	*<0.001*	1.731	1.688-1.776	*<0.001*	1.346	1.288-1.406

OR, odds ratio; CI, confidence interval. Logistic regression analysis was used.

Prediction of ALL susceptibility

Logistic regression analysis was conducted for prediction of ALL susceptibility, using age, gender, MDA, CAT, SOD and GSH. Higher MDA and GSH, lower CAT and SOD were associated with risk of ALL in univariable analysis. However, in multivariable analysis, Higher GSH and lower SOD were considered independent predictors of ALL susceptibility.

Acute lymphocytic leukemia (ALL) is a B or T lymphoblast malignancy characterized by uncontrolled proliferation of abnormal, immature lymphocytes and their progenitors, which leads to the replacement of bone marrow elements and other lymphoid organs, resulting in the typical ALL disease pattern. It accounts for approximately 2% of lymphoid neoplasms diagnosed in the United States. [26]

Because ALL is primarily detected in children and young adults, the current study sought to evaluate a number of biochemical indicators and oxidative stressors associated in ALL development. The current study was a case control study that included 100 patients with ALL who were recruited from an oncology outpatient pediatric clinic at Mansoura University Hospitals. The present study was conducted on 100 cases with ALL.

The present study was conducted on 100 cases with ALL. Their mean age was 9.62 years, ranged from 1 to 17 years. They were 64% males and 36% females. The mean weight of all studied cases was 31.54 kg, and ranged from 14.0 to 46.0 kg. In same line with Silva et al. [27] who had examined 577 cases with ALL, most patients were male, with a schooling period of 1–4 years, ALL patients were mostly in the age group ≤10 years (325 cases), followed by 122 patients aged 11–20 years, with all other age groups accounting for <10% of cases 172 (29.80%) of ALL.

Regarding the biochemical markers among patients with ALL. Elevated levels of transaminases (ALT, AST) were common at the initial stages of ALL and caused due to the liver injury by the leukemic cell infiltration, since Rasool et al. [28] revealed that in cases with ALL, alanine aminotransferase (ALT) was 86±36.07, aspartate aminotransferase (AST) was 56.08±18.6, alkaline Phosphatase *(*ALP) was 377.95±34.76, creatinine was 3.13±0.27, potassium (K) was 6.02±0.93, calcium (Ca) was 7.49 ± 1.06. sodium (Na) was 167 ± 12.06, magnesium (Mg) was 1.41 ± 0.21, Phosphate was 5.75 ± 0.50, uric acid was 10.35 ± 1.49, indicating injury of organs especially liver and kidney.

The maintenance of normal cellular homeostasis, regulation of fluid, electrolyte balance, and blood pressure (BP), requires sodium as an essential nutrient. Its role is crucial for maintaining extracellular fluid (ECF) volume because of its important osmotic action and is equally important for the excitability of muscle and nerve cells and the transport of nutrients and substrates through plasma membranes. [29]

Besides that, when the cell fluid imbalances, potassium increases in the blood as a result of moving from inside to outside ells, which leads to the patient's feeling of fatigue.

The current study revealed that ALL showed significantly higher Na and K ($p < 0.001$) when compared to the control group. This could result in Osmotic pressure of intra-cellular fluid, which necessitates the release of water from the cells and the occurrence of their dehydration (cellular dehydration), which in the end disrupts their function.

Moreover, Cell death and degradation processes depend on the concentration of potassium within them, where lack of

concentration helps to decompose cells. Increasing it can prevent decomposition enzymes from working.

According to Liou et al. [30] High levels of reactive oxygen species (ROS) were reported as key contributors to several malignancies specifically acute lymphoblastic leukemia (ALL)**.** Thus, MDA production is accelerated by lipid oxidation results in oxidative stress in the form of lipid peroxidation.[31]

Regarding the oxidative stress and antioxidants markers among ALL patients, the current study revealed that ALL showed significantly higher malondialdehyde (MDA), glutathione (GSH), significantly lower superoxide dismutase (SOD) and catalase (CAT), when compared to control group.

Our results are in agreement with other study, which revealed that significantly higher MDA, significantly lower SOD and catalase in ALL when compared to control Rasool et al.[28] Some studies have shown that oxidative stress caused by the imbalance between the generation of free radicals ROS and the antioxidant defense systems can activate various transcription factors, further affecting their transcriptional pathways. Oxidative stress plays an important role in the occurrence, development, treatment, and prognosis of leukemia. [32]

According to the findings of this study, MDA levels was significantly high in ALL patients compared to controls, which is consistent with Battisti et al.[33] Otherwise, increasing of MDA levels in ALL patients showing that oxidative stress is present as well as the plasma antioxidant state which is, weakened.[34]

Malondialdehyde high levels were also reported by Mahmoud et al. [35] as a lipid peroxidation (LPO) indicator in ALL patients. It means that the large increase in LPO in ALL patients, possibly as a result of OS, is caused by increased free radicals in accordance to the research of Rajeshwari et al.[36] MDA high levels in ALL patients means that the status of serum antioxidants are depleted. Besides that, MDA serum levels act as a predictive and diagnostic biomarker for leukemia, indicating disease progression.[34]

The results approved the hypothesis that malignant cells have strong free radical reactions; thus, they could link between high levels of oxidative damage and low antioxidant activity. The supposition that malignant cells accumulate excessive amount of ROS and there is a link between ROS activity and cancer development which was supported by this investigation as well as other study of Mannan et al.**,**[37] which confirm our current findings.

Decreasing the activity of antioxidants such as super dismutase (SOD) and catalase (CAT) may collaborate to the production of free radicals.[38] The present study suggest that SOD and CAT could intermediate with the direct removal of free radicals and the homeostasis of biological state. In accordance to such results, Nishiura et al. [39] and Sun et al.[40] reported significant SOD serum activity in acute leukemia. While, Nishiura et al.[39] discovered that a decline in SOD levels in the blood could associated with leukemia regression. This because of SOD is protecting cells from oxidative stress at low levels by catalyzing superoxide anion formed in the cell, while at higher, it acts as a peroxidase, by boosting hydrogen peroxide synthesis and causing cell injury. In contrast to the findings of this study, other one found that SOD and CAT levels were high in ALL patients compared to control group.[35]

On the other hand, Glutathione activity in this study was significantly high in ALL patients. This finding is in congruent with Mahmoud et al., [34] who discovered that GSH levels were higher in ALL patients versus controls. This outcome is in the line with the finding of, Hsiao et al. [40] who observed that, there is a relationship between GSH and hepatocellular carcinoma (HCC). According to the study, GSH levels were significantly higher in HCC tissue than in adjacent normal tissue. In agree with our findings, ALL patients in comparison to healthy people, had significant different adjustments in antioxidants defense mechanism and elevation in ROS generation, this indication supports the supposition that the cancer or malignant cells formed a lot or ROS and there is a correlation between ROS and cancer development, as found by Liou and Storz**.**[30]

Furthermore, glutathione contributes the DNA synthesis and heeling. GSH reducing in leukemia refers to the depleting of non-enzymatic antioxidant whish is, GSH. According to Li et al.,[42] immunological non-responsiveness to antigenic stimulation by stimulator cells could be because of GSH deficiency. GSH deficiency is a potential agent that acts as a free radical scavenger. Otherwise, elevation of GSH in leukemia blasts may be associated to the drug resistance used in the chemotherapy of ALL patients.

More studies are needed to determine the origin and species of ROS formed by leukemic cells, in addition to, whether therapeutic of ROS were generated by normal cell metabolism or by the malignant cell generation. The truth that OS inactivates antioxidant enzymes, resulting in causing LPO and protein carbonylation, might be used to find an efficient and cost-effective way to increase endogenous antioxidant production as well as incorporate antioxidants into nutrition to help neutralizing free radicals which excrete by cellular metabolisms.[41]

Because of cancer initiating in the operating system, this might be a good preventive for many leukemias. It's encouraging to note that, certain oxidative-process based treatments are presently being investigated in clinical trials. More studies are needed to more understand of the efficacy, long-term consequences, and the hazards associated with the use of these drugs. [43]

Recommendations of the present study are several, firstly, a large size of samples is needed for more confirmation. Secondly, we recommend to Investigate the interaction between sex or age and other variables**.** In general, more research is needed to confirm the results and to elucidate the underlying mechanisms.

In conclusion MDA, CAT, SOD and GSH showed high accuracy for discrimination between ALL cases and controls. Higher GSH and lower SOD were considered independent predictors of ALL susceptibility. In addition to elevated Na and K electrolytes may contribute in developing acute lymphoblastic leukemia by effecting the cellular homeostasis.

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