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Activity of Some Antioxidant Enzymes in Patients with Chronic Lymphocytic Leukemia

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Abstract*: The objective of the present study was to estimate the activities of antioxidant parameters such as plasma catalase (CAT), erythrocyte lysate reduced glutathione (GSH) and superoxide dismutase (SOD) as well as determination of plasma malondialdehyde (MDA) and total antioxidant capacity (TAO) in chronic lymphocytic leukemia (CLL) patients. Additionally, it was the determination of their correlation with CLL progression and their prognostic role in CLL outcome. This study includes 41 newly diagnosed, untreated CLL patients. Plasma CAT activity, erythrocyte lysate GSH concentration and also plasma MDA concentration were significantly higher in CLL patients when compared to normal subjects. Concerning to clinical outcomes of CLL patients, MDA concentration was significantly higher in patients suffered from lymphocytic doubling and those who progressed to more advanced stage. GSH concentration was significantly higher in those who suffered from lymphocytic doubling. The increased levels of CAT activity, MDA, and GSH concentrations indicate the higher oxidative stress levels in CLL patients. Oxidative stress stimulates the antioxidant defense systems. Additionally, the higher levels of plasma MDA shows the higher levels of lipid peroxidation. Therefore, antioxidants play a vital and an important role as a protective system against these free radicals and preventing the risk and complications of blood cancer of CLL disease.*

Keywords*: Chronic lymphocytic leukemia, catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant capacity (TAO).*

"1. Introduction"

Chronic lymphocytic leukemia is a type of neoplastic disorders which characterized by the progressive agglomeration and clonal development of proportionately mature malignant lymphocytes with increased proportion of cells being inhibited in the non-proliferative G0/G1 phase of cell cycle [1]. One of potential mechanisms for defective and abnormal apoptosis could be irregular oxidative stress which influences tumor growth and its clinical progression [2].

Reactive oxygen species (ROS) explained as active chemical species containing oxygen and as oxidizing agents with high reactive chemical characteristics [3]. They are widely generated endogenously in biological systems during cellular metabolic activities; most of them are generated in low levels, and removed by antioxidant systems during normal physiological conditions [4]. They have the ability to cause various types of tissue injury and damage cellular components in a variety of human diseases subsequently; the increased ROS can result in cell death by necrosis or by apoptosis suggesting chemical properties for killing malignant cells through mechanisms of free radicals [5].

Oxidative stress defined as the result of imbalances in antioxidant defence due to increased generation of free radicals which result in oxidative damage and so increased lipid peroxide levels [6]. Oxidative stress has a significant role in CLL pathogenesis, implicated in carcinogenesis, and progression of haematological malignancies (HM) disorders [7,8]. The gathering of oxidation products result in genomic instability, indicating that oxidative stress participates in signaling regulation of cells and plays an important function in proliferation of cells [9].

In CLL, malignant cells are very active in the generation of reactive oxygen species compared to normal cells and exist beneath inherent oxidative stress subsequently, they revealed to injury by agents generating ROS [10]. To control flux of reactive nitrogen species (RNS) and ROS and also, counteract their toxic effects, human aerobic cells and biological fluids of living systems improve their antioxidant systems that limit their production [11]. Therefore, living cells not only accommodated to a co-existence with free radicals but also, they advanced several antioxidant defense systems for the beneficial use of free radicals in a variety of physiological functions [12].

Antioxidants are vitamins, minerals, and enzymes, works against oxidation process to protect lipids from peroxidation and to protect the body cells and tissues from damaging effects which produced by free radicals [13]. They present at varying concentrations in food matrices which exist in plants or animals and from industrial working and external sources, directed for preventing decay of food components and premature oxidation [14]. The measurement of oxidative stress biomarkers and activities of antioxidant enzymes in CLL patients are beneficial as accurate prognostic markers which permit early treatment of these patients [6].

Therefore, the purpose of this study was to estimate antioxidant activities such as plasma catalase, erythrocyte lysate superoxide dismutase, reduced glutathione, plasma malondialdehyde levels and total antioxidant capacity as indirect representative markers for oxidative stress in chronic lymphocytic leukemia patients to achieve extensive view of antioxidant defense systems of CLL patients. Additionally, it was the determination of their correlation with CLL progression and their prognostic importance in CLL outcome.

"2. Subjects and Methods"

This study comprises 41 newly diagnosed, untreated, and asymptomatic confirmed typical CLL patients collected in the period from September 2013 to September 2015 from Mansoura Oncology Center (MONC), Mansoura University, Egypt. A written informed consent was provided and obtained from every participant patient and control prior to their enrollment and recruited into this study according to the guidelines of Committeé of Medical Ethics of Mansoura University Hospitals. This reported study protocol was examined and confirmed by the local ethics Committeé on Human Research at Mansoura University, Egypt.

CLL patients comprised 34 males (82.9%) and 7 females (17.1%) with ages ranged from 45-84 years old (the median age 59 years). In addition, 25 control subjects of matched age and sex comprised 18 males (70%) and 7 females (30%) with ages ranged from 52-62 years (the median age 56.5 years). CLL diagnosis was established clinically, biologically, morphologically by applying clinical and laboratory criteria, bone marrow examination and surface marker criteria according to international CLL workshop criteria (IWCLL) [15] and guidelines of NCI Working Group Criteria [16], and then approved by the flow cytometric immunophenotyping analysis for CD5, CD19, and monoclonal immunoglobulin expression [17].

CLL patients classified at diagnosis according to Rai and Binet staging systems [18, 19] for assessing prognosis. According to Binet system: A stage (early stages or low-risk category) included 12 (29.3%) cases, B stage (intermediate- risk category) included 18 (43.9%) cases and C stage (advanced stages or high-risk category) included 11 (26.8%) cases. According to Rai system: 0 and I stages (early stages or low-risk category) included 2 cases (4.9%), II stage (intermediate- risk category) included 28 (68.3%) cases, III and IV stage (advanced stages or high-risk category) included 11 (26.8%) of cases.

Seven ml of fresh whole peripheral blood samples were taken from each patient before any chemotherapeutic approach and from normal control subjects after 12 hours of fasting for the estimation of the activity of the studying antioxidants parameters. Hemolysed samples were excluded. Samples subsequently divided into: three ml of blood were placed in Heparinized tubes. After centrifugation at 4000 rpm for 15 min, plasma were collected and stored at –20°C up to the analysis was performed for the estimation of catalase activity according to Aebi`s method [20] at 510 nm, malondialdehyde concentration according to the method of [21] at 534 nm, and total antioxidant capacity according to the method of [22] at 505nm. 500µl of the remaining erythrocyte sediment were washed and lysed with 3ml normal saline 0.9% for four times to obtain erythrocyte lysate for estimation of superoxide dismutase activity according to method [23] at 25°C and 560 nm while, 200µl of the remaining erythrocyte sediment were washed and lysed with ice cold distilled water for determination of reduced glutathione according to method [24] at 405 nm. The prepared erythrocyte lysate were collected and stored at –20°C until analysis.

One ml whole blood was taken in sterile EDTA tube for complete blood count analysis by automated cell counter (CELL DYN RUBY, Laser instrument, USA). Three ml of the blood were

collected into plain tubes and the obtained serum was used for the chemical tests such as liver function tests (Alanine aminotransferse (ALT) according to the method of [25] and aspartate aminotransferase (AST) according to the method of [26], albumin according to the method of [27] and total bilirubin in accord with method [28], kidney function tests (creatinine according to the method of [29] and uric acid according to the method of [30] and lactate dehydrogenase (LDH) according to the method of [31] carried out on (BT 3500 instrument, Italy).

Statistical calculations were done by using excel program (Microsoft Office 2010) and Statistical Package for Social Science program (SPSS, Inc, Chicago, IL) version 20 [32].

"3. Results"

Table 1 shows that there were significant alterations of antioxidative systems in CLL patients when compared to control subjects. Plasma catalase activity, erythrocyte lysate reduced glutathione concentration and also, plasma malondialdehyde concentration were significantly higher in CLL patients when compared to control group (P=0.040, 0.048, 0.021 respectively) (see Figure 1).

Correlations between studied parameters versus each other and different haematological and biochemical parameters in CLL patients are showed in table 2 and 3. Total antioxidant capacity showed significant positive correlations with catalase, malondialdehyde, and total bilirubin (r=0.443, P=0.004; r=0.311, P=0.048; r=0.323, P=0.039, respectively). Catalase showed a significant positive correlation with malondialdehyde (r=0.304, P=0.043). Malondialdehyde showed a significant positive correlation with total bilirubin (r=0.329, P=0.036). Reduced glutathione showed a significant positive correlation with platelet count (r=0.395, P=0.041). Total antioxidant capacity showed a significant negative correlation with reduced glutathione (r=-0.390, P=0.046). Catalase and superoxide dismutase showed significant negative correlation with platelet count (r=-0.313, P=0.046; r=-0.329, P=0.036, respectively). Additionally, CLL patients showed a significant negative correlation between reduced glutathione and lactate dehydrogenase (LDH) (r=-0.325, P=0.038). Table 4 shows that no significant differences were found when comparing the studied antioxidants activities in CLL patients according to different clinical stages.

In this study, the comparison between studied antioxidants activity according to clinical outcome in CLL patients comprises lymphocyte doubling (LD) (defined as the progressive lymphocytosis with the increase of greater than 50% extra a 2-month period), progression to a more advanced stage (defined as the period elapsed from study entry or diagnosis until objective disease progression, first line treatment, last follow-up, or death), and mortality (number of deaths of the total size in a particular population during the entire period of the study) (see Table 5). Results showed that malondialdehyde concentration was significantly higher in patients suffered from lymphocytic doubling (LD) and those who progressed to more advanced stage (P=0.008 for both), while reduced glutathione concentration was significantly higher in those who suffered from lymphocytic doubling (P=0.041).

"4. Discussion"

CLL is a non-specific neoplastic chronic disease which increases reactive oxygen species production [33]. CLL cells are susceptible to alterations of oxidative stress and antioxidant enzymes [34]. Both the alteration of antioxidant enzymes and increase ROS production has been described to contribute tumorigenesis [35]. Disturbances in metabolism of oxidative stress act as a common feature for transmute tumor cells [36].

In our study, plasma catalase (CAT) activity was statistically significant higher in CLL patients when compared to control group (P=0.040). This is in agreement with Zaric et al. 2011 [37]. On the other hand, the present results are in disagreement with some studies in lymphocyte lysates catalase [1, 37] and in erythrocyte catalase in CLL patients [38, 39].

B-CLL lymphocytes express and release catalase as the most important enzyme in detoxification of hydrogen peroxide more than healthy lymphocytes [40]. Interestingly, B-CLL patients with advanced disease presented significantly higher plasma catalase activity leading to decrease of plasma hydrogen peroxide (H_2O_2) levels. CLL progression was associated with significantly higher plasma catalase activity and the increased plasma catalase activity in patients with CLL reveals the stimulation of antioxidant defense systems [41].

Erythrocyte catalase levels usually a meaningful significantly decrease in leukemias only in untreated CLL patients causing the accumulation of excess amounts of hydrogen peroxide (H_2O_2) in tumor cells suggesting an association found between erythrocytes catalase activity and the kind of chronic leukemia such as CLL or chronic myelogenous leukemia (CML). Furthermore, catalase plays a significant role in types of chronic leukemias and acts as antioxidant enzyme in erythrocytes [42]. This indicates the function of antioxidant enzymes in the avoidance of cancer [43].

In the present study, plasma malondialdehyde concentration which a marker of oxidative stress was statistically significant higher in CLL patients when compared to normal subjects (P=0.021) leading to increased lipid peroxidation levels indicating the instability found between antioxidants and oxidants in plasma of patients with CLL. The intracellular malondialdehyde level acts as a marker of disease evolution. Our results are in agreement with [41, 44].

The process of lipid peroxidation defined as the oxidative alterations of polyunsaturated fatty acids which significant for normal functions of mammalian cells. Lipid peroxidation of cellular components plays a significant role in the pathogenesis of numerous diseases and carcinogenesis. Malondialdehyde is a lipid peroxidation end-product, stimulated by ROS and associated with the extent of lipid peroxidation [45]. On the other hand, several writers indicated that MDA represents as co-carcinogenic agent and tumor promoter due to its high inhibitory and cytotoxicity effect on protective antioxidant enzymes [46].

CLL patients demonstrated an impaired antioxidant systems that lead to accumulation of reactive oxygen species and free radicals which indicated by higher MDA levels which might be a proper marker of oxidative stress and suggestive steps for the process of lipid peroxidation. Interestingly, CLL progression correlated with significantly higher plasma MDA and CAT activity as the duration of the disease increased in years throughout CLL progression time [2, 47]. The increased lipid peroxide levels are suggestive for moderate oxidative stress due to insufficient response of tissues to oxidative stress by increasing the antioxidant defense systems [6].

The higher lymphocyte malondialdehyde levels of B-CLL patients than healthy subjects might not be only a consequence of increased production but also, a failure of the antioxidative enzyme defense system such as the decrease of SOD and CAT activities specifically lymphocytes lysates glutathione peroxidase (GPx) causing that superoxide anion and hydrogen peroxide accumulated inside tumor cells, so lipid peroxidation was importantly and significantly increased in malignant cells of CLL patients leading to increased plasma MDA concentration which accompanied by the rise of the MDA in supernatants of lymphocytes cultures supporting a predominant vulnerability of lymphocytes to oxidative stress status in CLL [1,48].

Moreover, erythrocyte lysates reduced glutathione (GSH) concentration was statistically significant higher in CLL patients when contrasted to control subjects (P=0.048). Our results are in accordance with [34, 49]. Unlike us, Ortin et al. 2012 reported that erythrocyte reduced glutathione (GSH) was significantly decreased in CLL patients and revealed a probably malfunctioning of erythrocytes GSH turnover system and instability of prooxidant/ antioxidant equilibrium in early stage CLL patients. Additionally, our results are in disagreement with other studies in lymphocytes, serum, and plasma reduced glutathione (GSH) concentration in CLL patients [39].

The increase in reduced glutathione levels was directly proportional to disease duration as a consequence of increase tripeptide synthesis depending on the increased lymphocytes GPx activity in CLL [34, 49]. Also, it is associated with accompanying increase of glutathione reductase (GRD) which converts GSSG back to GSH however GRD activity did not alter in CLL cells [50].

Erythrocyte lysates superoxide dismutase (SOD) activity have not a significant differences in CLL patients when contrasted to normal subjects (P=0.783) causing that hydrogen peroxide and superoxide anion accumulated in tumor cells that is one mechanism caused for the increased plasma and serum MDA concentration in B-CLL patients when compared to control subjects [48]. Our results are in accordance with [37, 41]. [1], showed a slightly increase in blood plasma superoxide dismutase activities for both early and advanced stages of CLL patients but not significantly different from controls so, there was no statistically importance.

In the case of severe oxidative stress, there is an intensive production of ROS that overcome antioxidant capacity of the cells and this phenomena consequently leads to the breakdown of all antioxidative defense machineries that is manifested as no statistically significant differences of SOD activities [1]. Antioxidant defence system can be impaired as a result of irregularity in antioxidative metabolism because of cancer process [51].

Our results demonstrated that there was no significant differences of plasma total antioxidant capacity (TAO) in CLL patients when compared to control subjects (P=0.491). This is in agreement with Papageorgiou et al. 2005 [52], in case of children patients with HM prior to commencement of chemotherapy however, on commencement of chemotherapy, there was a progressive decline in TAO of children patient with haematological malignancies (HM). The decrease in total antioxidant capacity (TAO) was attributed to the oxidant stress of chemotherapy.

Table 2, shows correlations between antioxidants versus each other in our CLL patients demonstrated that total antioxidant capacity (TAO) showed

significant positive correlations with catalase (CAT) and malondialdehyde (MDA) (r=0.443, P=0.004; r=0.311, P=0.048 respectively) and a significant negative correlation with reduced glutathione (GSH) (r=-0.390, P=0.046). Catalase (CAT) showed a significant positive correlation with malondialdehyde (MDA) (r=0.304, P=0.043). These correlations indicated the presence of oxidative stress in cells of CLL patients and the levels of antioxidants are increased to remove the excessive production of oxygen free radicals. On the other hand, Bakan et al. 2003 [43] reported that GSH levels in serum was negatively correlated with serum MDA in CLL patients of advanced stages (III + IV) indicating that glutathione is used to remove the increase of free radicals because of severe wickedness.

Ortin et al. 2012 [39], showed that global antioxidant capacity correlated negatively with SOD and GPx levels and positively with erythrocyte and plasma values of GSSG, GSSG/GSH level, and pro-oxidant thiobarbituric acid reactive substances in CLL patients indicating the increase of score oxidative stress (SOS) in CLL patients who present a compensatory mechanism of the body against a free radical attack and respond to the attack of the radicals [53, 54].

Moreover, correlations between studied antioxidants activity and different haematological and biochemical parameters in CLL patients showed significant positive correlations between total bilirubin versus TAO and malondialdehyde (MDA) (r=0.323, P=0.039; r=0.329, P=0.036, respectively). Also, significant negative correlations between platelet count versus catalase (CAT) and superoxide dismutase (SOD) (r=-0.313, P=0.046; r=-0.329, P=0.036, respectively) and a significant positive correlation between platelet count versus reduced glutathione (GSH) (r=0.395, P=0.041). Additionally, CLL patients showed a significant negative correlation between lactate dehydrogenase (LDH) versus reduced glutathione (GSH) (r=-0.325, P=0.038).

Gonzales et al. 1984 [55] investigated the relationship between red blood cells SOD activity and the different parameters such as hemoglobin (Hb) concentration, erythrocyte count and reticulocyte count in various diseases such as CLL patients. The correlation coefficient (r) obtained between SOD levels, hemoglobin concentration, erythrocyte count and reticulocyte count but, these values are not statistically significant in all cases indicating that SOD activity is not influenced by secondary factors suggesting that SOD level reflects an intrinsic increase of SOD activity in the RBC in each group of patients. Furthermore, they indicated the failure of association between the increased superoxide dismutase activity and various clinical and biological parameters. The increase of SOD activity is due to interrupted gene expression in the stem cells and chemotherapy effect result in normalization of SOD activity [55].

Concerning to total antioxidant capacity (TAO) with respect to age of CLL patients, [56], reported that CLL patients showed low antioxidant capacity values at diagnosis in CLL patients over 60 years old due to the natural decrease in antioxidant capacity (TAO) with increasing age. Also, Poongothai et al. 2004 [57] reported that there was a significant decrease of total antioxidant status in male not in female with respect to age in leukemia patients such as CML, AML, ALL. However, with respect to sex, total antioxidant status was higher in males that in females, suggesting that genetic difference discovered in management of oxidative status and explained the significant difference of sex in superoxide dismutase activity only in chronic myelogenous leukemia (CML) patients [58]. [59], demonstrated that total antioxidant capacity (TAO) does not differ significantly with age in a study of 422 males with an age range 19.2-89 years.

Ghalaut et al. 1999 [60] reported that there was no correlation observed between lymphocyte glutathione (GSH) levels and the reduction of total cell count (TLC) in acute myeloid leukemia (AML) patients. This was similar to that of [61], who showed that there was no significant association between lymphocyte GSH concentration and laboratory/clinical parameters. They also deduced that it is not the only determinant for response to chemotherapy. Moreover, our data showed that there were no significant differences when comparing the studied antioxidants activities in CLL patients according to different clinical stages neither (Binet stage nor Rai stage). This result is in accordance with [55], who showed that there was no relationship between superoxide dismutase activity and expansion or stage of CLL and the existence of inflammatory symptoms.

Unlike us, Zelen et al. 2007 [1] reported that a significant reduce in lymphocytes SOD, CAT, and GPx activities found in both groups of early and advanced stages of CLL patients compared to control subjects and the decrease in activities is progressively enhanced by the advanced disease stage and so, the lower enzyme activity is directly proportional to the increased stage of the disease. However, plasma catalase activity was increased in advanced CLL stage compared to normal healthy subject. Zelen et al. 2010 [41] indicated that there was significant changes of plasma catalase activity among CLL groups (control vs. B+C stages and A stage vs. B+C stages) and they showed that the lowest value of plasma CAT activity was detected

in the control group and higher value was seen in the A stage B-CLL patients while the highest value of plasma CAT activity was detected in the plasma of the B + C stages of disease.

Djurdjevic et al. 2009 [2] showed significant differences between supernatants of cultured leukemic and normal lymphocytes MDA level in stage B and C of CLL patients compared to controls. They reported that there was significantly higher supernatant lymphocyte malondialdehyde concentrations in B, C stages of patients group compared with the control group, indicating intensive lipid peroxidation process in these advanced stages (B and C stages). Although supernatant MDA level was slightly increased with disease progression, the difference was not significant and also, there were no differences found in MDA level of stage A CLL patients compared with control group or between both groups of CLL patients. Gaman et al. 2014 [56] reported that CLL patients showed high values of free oxygen radicals indicated a high ROS levels especially in advanced stages of disease (B and C stages) when malignant lymphocytes are very active in ROS production by a process exacerbated by aggressive chemotherapy.

Our data showed that malondialdehyde (MDA) concentration was significantly higher in patients suffered from lymphocytic doubling (LD) and those who progressed to more advanced stage (P=0.008 for both) and reduced glutathione (GSH) concentration was significantly higher in those who suffered from lymphocytic doubling (LD) (P=0.041). These results are in accordance with Zelen et al. 2007 [1] who showed that the analysis of antioxidant enzyme activities like lymphocytes SOD, CAT, and GPx in patients with CLL can be applied as good prognostic parameters for the disease effect. However, determination of protective enzyme activities in plasma of CLL patients is a poor prognostic factor since the presence of these enzymes in plasma may originate from other cell types than lymphocytes.

In conclusion, the identification and determination of antioxidant activities and adequate oxidative stress biomarkers of tumor cell metabolism could be functional for early diagnosis of CLL patients and assessment of tumor development. Additionally, they act as predictive factors and have prognostic relevance for prediction of clinical disease outcomes and progression of CLL disease. Therefore, antioxidants are suggested to be simultaneously evaluated in order to enhance the detection of CLL disease because, antioxidants play a vital and an important role as a protective system against these free radicals and preventing the risk and complications of blood cancer of CLL disease.

"5. Recommendations"

The determination of antioxidant enzyme activities should be applied at diagnosis of CLL patients and the supplementation of antioxidants should be recommended to improve the antioxidant enzyme activities and the quality of life in CLL patients. Additionally, the possibility of using antioxidants in combination with existing therapeutic strategies should be recommended to improve survival in CLL patients because treatment of CLL cells using antioxidants neutralize endogenous ROS and protect the immune system.

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Table 1. Statistical analysis of the activities of superoxide dismutase (SOD) and catalase (CAT) and the concentrations of reduced glutathione (GSH), malondialdehyde (MDA) and total antioxidant capacity (TAO) in chronic lymphocytic leukemia patients (CLL) compared with control group.

	Control	CLL
	N=25	N=41
GSH (mmol/L)	0.07	
Median	0.04 - 0.15	0.12
Range		0.04 - 0.25
P		0.048
TAO (mM/L)	1.59	
Median		1.48
Range	1.06 - 1.73	0.58 - 1.75
P		0.491
CAT (U/L)	384.50	
Median	222 - 513	564
Range		213 – 873
P		0.040
SOD (U/mL)	157.99	
Median	114.58 - 225.69	159.72
Range		114.58 - 225.69
P		0.783
MDA (nmol/ml)	3.46	
Median	2.50 - 5.29	5.28
Range		1.28 - 55.90
P		0.021

Significance probability $P < 0.05$.

Table 3. Correlations between studied antioxidants activity and different parameters in CLL patients.

CLL	GSH (mmol/L)		TAO (mM/L)		CAT (U/L)		SOD (U/mL)		MDA (nmol/ml)	
	r	P	r	P	r	P	r	P	r	P
Age (years)	-0.196	0.220	0.117	0.467	-0.104	0.517	0.145	0.366	0.004	0.980
Total leucocytic count ($X10^9$/L)	0.118	0.462	0.018	0.909	0.053	0.740	-0.019	0.905	-0.192	0.229
Hemoglobin concentration (g/dL)	0.037	0.818	0.055	0.733	-0.202	0.205	-0.064	0.693	-0.097	0.547
Platelet count ($X10^9$/L)	0.395	0.041	-0.163	0.309	-0.313	0.046	-0.329	0.036	0.018	0.913
Absolute lymphocytic count (X 10^9/L)	0.188	0.240	-0.032	0.843	-0.014	0.929	0.006	0.971	-0.183	0.252
Relative lymphocytic count (%)	-0.077	0.634	0.174	0.275	0.010	0.952	-0.059	0.714	0.116	0.470
Prolymphocytes (%)	0.101	0.531	-0.215	0.178	-0.079	0.623	-0.129	0.420	0.019-	0.905
ALT (U/mL)	0.020	0.900	0.225	0.158	0.152	0.342	-0.006	0.970	0.180	0.261
AST (U/mL)	-0.108	0.501	0.129	0.422	0.132	0.409	-0.023	0.888	0.240	0.131
Total bilirubin (mg/dL)	-0.160	0.318	0.323	0.039	0.270	0.088	0.245	0.122	0.329	0.036
Albumin (g/dL)	0.190	0.234	0.061	0.704	0.050	0.756	0.048	0.768	-0.223	0.162
Creatinine (mg/dL)	0.173	0.280	0.148	0.355	-0.103	0.521	0.137	0.391	-0.179	0.264
Uric acid (mg/dL)	0.031	0.847	-0.214	0.178	-0.165	0.303	0.120	0.454	-0.074	0.647
LDH (U/mL)	-0.325	0.038	0.221	0.166	-0.043	0.789	0.235	0.139	0.151	0.345

r: correlation coefficient .
ALT: Alanine aminotransferse. AST: Aspartate aminotransferase. LDH: Lactate dehydrogenase.

Table 4. Statistical analysis of the concentration of reduced GSH, TAO and MDA as well as the activities of CAT and SOD according to clinical stages in studied CLL patients.

CLL	Binet stage			Rai stage	
	A	B	C	0+I+II	III+IV
GSH (mmol/L) Median Range P	0.09 0.04 - 0.24	0.13 0.04 - 0.22	0.09 0.04 - 0.25 0.550	0.11 0.04 - 0.24	0.09 0.04 - 0.25 0.435
TAO (mM/L) Median Range P	1.48 0.85 - 1.74	1.54 0.58 - 1.75	1.12 0.68-1.71 0.295	1.54 0.58-1.75	1.12 0.68 - 1.71 0.145
CAT(U/L) Median Range P	409 233 - 873	564 213 - 762	682 276- 820 0.283	564 213 - 873	682 276 - 820 0.119
SOD (U/mL) Median Range P	184.02 114.58-225.96	145.30 114.58-225.69	184.02 114.58-222.22 0.525	157.99 114.58-225.96	184.02 114.58-222.22 0.813
MDA (nmol/ml) Median	5.21	5.61	5.28	5.44	5.28

Range P	1.39 - 55.90	1.28 - 10.30	1.59 - 12.39 0.956	1.28 - 55.90	1.59 - 12.39 0.860

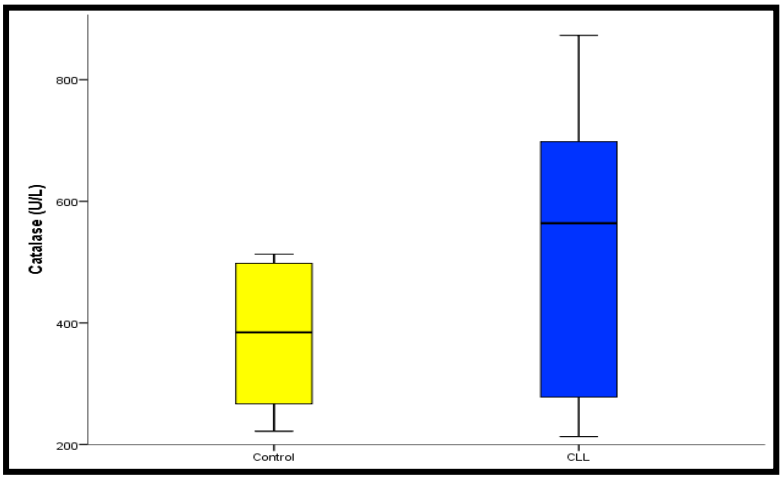
Significance probability P <0.05.

Table 5. Comparison between the concentration of reduced GSH, MDA and TAO, as well as the activities of CAT and SOD according to clinical outcome in CLL patients.

CLL	Lymphocytic doubling		Progression to a more advanced stage		Mortality	
	No	Yes	Stable	Progressed	Alive	Died
GSH (mmol/L) Median Range P	0.09 0.04- 0.25	0.14 0.04 - 0.24 0.041	0.10 0.04 -0.25	0.11 0.04 - 0.24 0.249	0.10 0.04 - 0.25	0.10 0.04 - 0.24 0.758
TAO (mM/L) Median Range P	1.51 0.58 - 1.74	1.48 0.85 - 1.75 0.510	1.51 0.58 - 1.74	1.48 0.68 - 1.75 0.499	1.52 0.58 - 1.75	1.07 0.68 - 1.72 0.226
CAT (U/L) Median Range P	585.50 222 - 873	564 213 - 873 0.644	575.50 213 - 873	564 238 - 873 0.596	564 213 - 873	673 256 - 873 0.291
SOD (U/mL) Median Range P	154.51 114.6-225.9	159.72 114.6-225.7 0.910	168.41 114.6-225.9	159.72 114.6-222.2 0.811	159.72 114.6-225.9	156.25 114.6-222.2 0.791
MDA (nmol/ml) Median Range P	5.17 1.28-6.72	9.03 2.10-55.90 0.008	5.11 1.28 - 6.72	5.86 1.59 - 55.90 0.008	5.28 1.28 - 12.39	10.06 1.59 - 55.90 0.391

Significance probability P <0.05.

LD: Lymphocytic doubling.



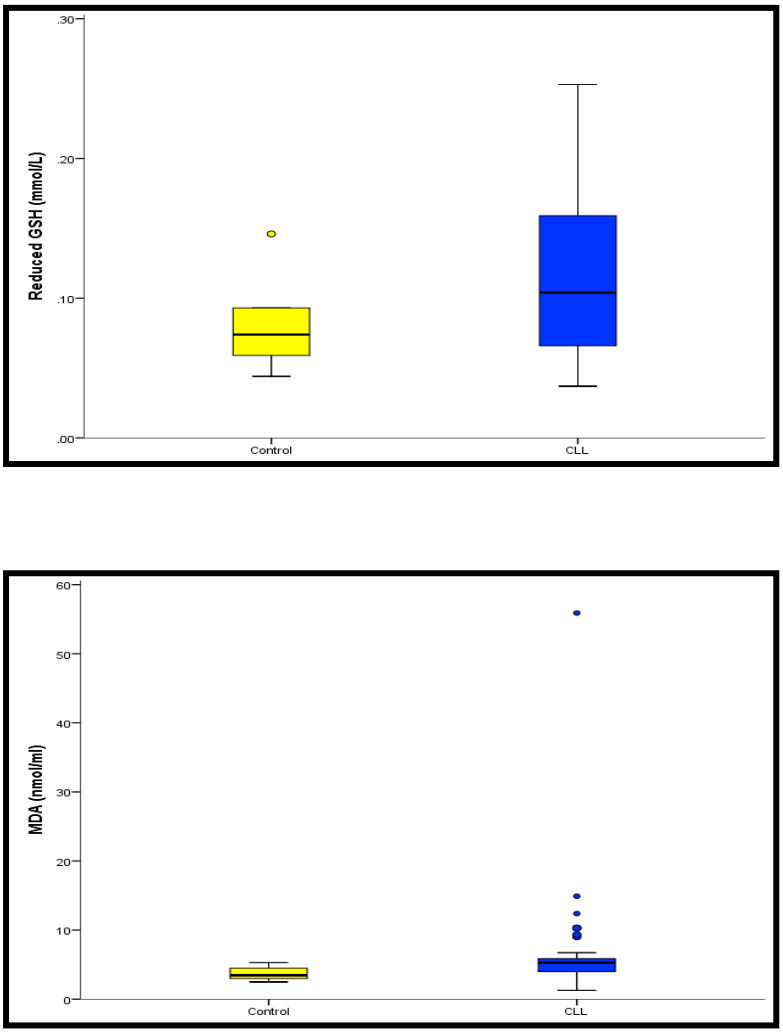


Figure 1. Comparison of the activities of CAT and the concentrations of reduced GSH and MDA in CLL patients and control subjects.