Evaluation of dynamic thiol-disulfide homeostasis and ischemia-modified albumin levels in patients with chronic lymphocytic leukemia

Huseyin Erdal ,[1] Rafiye Ciftciler,[2] Sibel Cigdem Tuncer,[3] Oguzhan Özcan[4]

[1]Medical Genetics, Aksaray University, Aksaray, Turkey
[2]Hematology, Aksaray University, Aksaray, Turkey
[3]Biochemistry, Aksaray University, Aksaray, Turkey
[4]Biochemistry, Hatay Mustafa Kemal University, Hatay, Turkey

Correspondence to
Dr Huseyin Erdal, Medical Genetics, Aksaray University, Aksaray, Merkez, Turkey; herdalyfa@gmail.com

Accepted 10 August 2022

Check for updates

© American Federation for Medical Research 2022. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Erdal H, Ciftciler R, Tuncer SC, *et al*. *J Investig Med* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jim-2022-002568

ABSTRACT

This is the first study to evaluate both the dynamic thiol-disulfide homeostasis and ischemia-modified albumin (IMA) levels in patients with chronic lymphocytic leukemia (CLL). Twenty-nine patients with CLL and 20 controls were included in the study. The dynamic thiol-disulfide balance was determined by the newly developed colorimetric method by Erel. IMA levels were determined by the cobalt binding test. We found that total antioxidant status levels were lower while total oxidant status (TOS) and oxidative stress index (OSI) levels were significantly higher in patients with CLL than controls. Moreover, native and total thiol levels were found to be statistically significant between the study and control groups ($p<0.001$), whereas no statistically significant difference was noted for IMA levels ($p=0.365$). A negative correlation was observed between native and total thiol levels, leukocyte, lymphocyte, and TOS. Total bilirubin showed positive correlation with direct bilirubin and alkaline phosphatase. In addition, IMA levels showed a positive correlation with OSI. This study highlights measurement of native and total thiol and IMA levels in patients with CLL for the first time. Dynamic thiol-disulfide homeostasis may contribute in the pathophysiological mechanism, and follow-up to disease in patients with CLL.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults.
⇒ There is no study to investigate thiol-disulfide homeostasis and ischemia-modified albumin (IMA) levels in patients with CLL.

WHAT THIS STUDY ADDS

⇒ The present study shows that oxidative stress increased significantly in patients with CLL.
⇒ These findings strongly favor the impairment of oxidant-antioxidant balance.
⇒ IMA levels were found to be high in patients with CLL.
⇒ Native and total thiol levels were found to be significantly lower in patients with CLL.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results indicate that determination of dynamic thiol-disulfide levels can serve as an aid for the diagnosis and follow-up the disease, particularly for the early identification of CLL.
⇒ The increase in IMA level may be associated with oxidative stress and functional reduction of the antioxidant defense system.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is one of the most common cancer types in the world, with an incidence of 4–5 per 100,000 people. The incidence rate of CLL among men is higher than the one among women. The average diagnosis age among men is about 72 years old.[1] The clonal growth and accumulation of CD5-positive B cells in the blood, bone marrow, and lymph nodes are among the most prominent features of CLL.[2] Moreover, one of the strongest genetic predispositions of hematological malignancies is CLL. A positive family history of CLL affects around 10% of those who get the disease. Living in the countryside or exposure to chemicals, family history of disease, and widespread infection carries a high risk for the development of CLL.[3] Ten per cent of patients with CLL have 17p deletion on the chromosome where the p53 gene is located, and 30% have a mutation in the p53 gene. Genetic changes that occur in this way may lead to an increase in the generation of reactive oxygen species (ROS) in vivo, which may further explain the link between loss of p53 function and poor clinical outcomes and drug resistance in 17p-deleted CLL cells.[4] While determining prognosis for patients with CLL, the versatile risk profile remains a challenge. As a result, numerous efforts are required to uncover novel prognostic indicators that might be used to create a risk classification system for patients with CLL at the time of diagnosis. Oxidative stress (OS) biomarkers may provide a new frontier in terms of prospective candidates for improving the predictive capacity of laboratory techniques used to determine the prognosis of patients with CLL. OS occurs with the abnormal increase of free radicals in the cell and the deterioration of the oxidative balance as a

J Investig Med: first published as 10.1136/jim-2022-002568 on 29 August 2022. Downloaded from file:/ on April 19, 2024 by guest. Protected by copyright.

result of insufficient antioxidants in the body,[5 6] which plays a critical role in the occurrence of many common diseases such as chronic inflammation, diabetes, and cancer.[7–9] The primary target of ROS produced in the cell as a result of OS is thiol groups. Thiols are found in amino acids that are found in the structure of proteins and contain sulfur in their composition. Thiols are exposed to oxidation reactions by oxidants formed under OS and turns into disulfide structures.[10 11] Disulfide bond, which is a covalent bond, is also called S-S and disulfide bridge. Disulfide structures (S-S), which are formed as a result of OS are reduced to thiol groups again, and thiol balance is achieved.[12] In many studies, the measurement of dynamic thiol disulfide balance has been shown as an indicator of increased free radical levels in various diseases.[13–16] The increase in OS causes free radical damage at the N-terminal end of albumin molecules and decreases the binding capacity of albumin. The modified form of albumin is called ischemia-modified albumin (IMA) and is used a responsive marker of OS.[13] To the best of our knowledge, this is the first study to evaluate both dynamic thiol-disulfide hemostasis and IMA levels in patients with CLL.

MATERIALS AND METHODS

Patient and control groups

Twenty-nine patients with CLL under care at the hematology outpatient clinic of Aksaray University Training and Research Hospital were included in the study. The control group consisted of 20 healthy individuals with no statistically significant difference in terms of gender and age. Demographic information of the study and control groups were collected from the hospital automation system. Hematological diseases and malignancies other than CLL were extricated from the current study.

Sample collection of the study

Fasting blood samples were taken into EDTA tubes from the patients with CLL and control groups. Subsequently, samples were centrifuged at 1500 × g for 10 min separated into Eppendorf tubes and stored in a refrigerator at −20°C until the time of the assay.

TAS and TOS measurements

Total oxidant status (TOS) and total antioxidant status (TAS) levels were measured spectrophotometrically based on method developed by Erel.[17] The measured data were expressed as µmol Trolox equivalent per liter for TAS and mmol H_2O_2 equivalent per liter for TOS. Then, oxidative stress index (OSI). OSI (arbitrary unit)=TOS (µmol H_2O_2 Eq/L)/TAS (µmol Trolox Eq/L)×100 were calculated.

Determination of thiol-disulfide levels

Thiol-disulfide levels were determined by automated spectrophotometric method as previously described by Erel and Neselioğlu.[12] Briefly, disulfide bonds are reduced to thiol groups with sodium borohydride. To prevent reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid, unused sodium borohydride was consumed and removed with formaldehyde. Disulfide levels were determined by dividing the difference obtained by subtracting native thiols from total thiols by two.

Table 1 Demographic features of participants

Parameter		CLL (n=29) N %	Control (n=20) N %	P value
Gender	Male	13 (44.8%)	11 (55%)	0.56*
	Female	16 (55.2%)	9 (45%)	
Age (year)		66.1±9.2	62.5±12.4	0.47†

*χ^2 test.
†Student's t-test.

Measurement of IMA levels

IMA levels were analyzed by using albumin cobalt binding test developed by Bar-Or *et al*.[18] The method is based on the binding ability of reduced cobalt ions (Co^{2+}) alongside addition of known amounts of exogenous Co ($CoCl_2$) to serum samples. Due to ischemia, altered albumin binds less to Co(II) and excess (unbound) Co^{2+} produces a colored structure with dithiothreitol measured spectrophotometrically at 480 nm. The results were expressed as absorbance units.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) V.22 (SPSS, Chicago, Illinois, USA) program was used for statistical analysis. The normal distribution of the data was determined by the Shapiro-Wilk test. The mean differences between two independent groups were compared with the Student's t-test. Comparison of values that did not fit the normal distribution was done with the Mann-Whitney U test. Spearman's correlation test was used for correlation analysis. The p value <0.05 was accepted as statistical significance level.

RESULTS

This study consisted of 49 patients, 29 in the CLL group and 20 in the control group. There was no significant difference between the groups in terms of age and gender (table 1).

In the CLL group, 13 (44.7%) of the patients were male and 16 (55.2%) were female. In the control group, 11 (55%) of the participants were male and 9 (45%) were female. Among the hemogram parameters, platelet, leukocyte, lymphocyte, monocyte, lactate dehydrogenase, direct bilirubin, and sedimentation were found to be significantly different between the two groups (table 2).

Table 2 Laboratory parameters of study and control groups

Parameter	CLL mean (n=29) (min–max)	Control mean (n=20) (min–max)	P value
Lymphocyte (10^3µL)	37.08 (1.13–224)	1.65 (0.97–2.81)	0.001*
Leukocyte (10^3µL)	43.99 (3.9–232)	7.83 (1.7–28.9)	0.001*
Monocyte (10^3µL)	1.09 (0.06–7.48)	0.43 (0.16–1.03)	0.023
LDH (U/L)	229.3 (160–468)	202.6 (135–490)	0.039
PLT (10^3µL)	183.9 (110–299)	229 (81–420)	0.036
Direct bilirubin (mg/dL)	0.10 (0.05–0.25)	0.13 (0.06–0.20)	0.047
ESR (mm/h)	11.2 (1–39)	18.45 (3–68)	0.027

*Mann-Whitney U test.
ESR, Erythrocyte sedimentation rate; LDH, lactate dehydrogenase; max, maximum; min, minimum; PLT, platelet.

J Investig Med: first published as 10.1136/jim-2022-002568 on 29 August 2022. Downloaded from file:/ on April 19, 2024 by guest. Protected by copyright.

Table 3 Thiol-disulfide homeostasis parameters of the study and control groups

Parameter	CLL mean (n=29) (min–max)	Control mean (n=20) (min–max)	P value
Total thiol (µmol/L)	399.4 (265–501)	483.1 (443–535)	0.001*
Native thiol (µmol/L)	348.8 (210–460)	444.3 (405–492)	0.001*
Disulfide (µmol/L)	25.3 (13–56)	18.4 (10–25)	0.007*
Disulfide/Native thiol (%)	7.76 (3.3–16.5)	4.1 (2.2–5.6)	0.001*
Disulfide/Total thiol (%)	6.53 (3.1–12.4)	3.8 (2.1–5.1)	0.001*
Native thiol/Total thiol (%)	86.9 (75.2–93.8)	92.3 (89.9–95.2)	0.001*
IMA (ABSU)	1.03 (0.34–3.98)	0.95 (0.47–3.54)	0.365
TAS (nmol Trolox/L)	1.34 (1.03–1.62)	1.48 (1.1–1.97)	0.104
TOS (µmol H_2O_2 Eq/L)	9.1 (5.7–16.8)	5.15 (7.2–3.1)	0.001*
OSI	0.67 (0.27–1.40)	0.35 (0.18–0.49)	0.001*

*Mann-Whitney U test.
ABSU, absorbance units; IMA, ischemia-modified albumin; max, maximum; min, minimum; OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status.

Native and total thiol levels were found to be significanlty lower in patients with CLL compared with the control subjects (p<0.001). However, disulfide levels were significantly higher in the patients with CLL compared with the control group (p<0.001). Disulfide/native, disulfide/total, and native/total levels were statistically significantly different between the patient and control groups (p<0.001). Moreover, TOS and OSI levels were also statistically significantly different between patient and control groups (p<0.001). However, TAS levels were not significanlty different between two groups (p<0.104). In addition, serum IMA levels were also not significantly different between patient and control groups (p=0.365, table 3).

Native and thiol levels showed negative correlation with leukocyte, lymphocyte, and TOS levels. In addition, it showed positive correlation with total bilirubin, direct bilirubin, and ALP. Besides that, IMA levels showed positive correlation with OSI (table 4).

DISCUSSION

We demonstrated that total and native thiol levels were significantly lower in patients with CLL than control subjects. However, disulfide levels were significantly higher in patients with CLL compared with the control subjects. Our study also revealed that IMA levels were not statistically significantly different in CLL groups compared with the controls. We also demonstrated that serum TAS levels were lower in patients with CLL group than healthy controls. However, TOS and OSI levels were significantly higher in patient group compared with the controls. In our study, we also evaluated the ratios of disulfide/native, disulfide/total, and native/total thiol levels between the control and patient groups and a statistically significant difference was found. This is the first study investigating thiol-disulfide and IMA levels as an oxidative marker in patients with CLL.

Thiol is an organic compound that consists of sulfur and hydrogen atoms attached to a carbon atom and plays a significant role in preventing OS formation in cells. Thiols are effective antioxidants that maintain the basic structure of proteins and protect cells and tissues from OS damage The majority of the thiol pool consists of albumin thiols and protein thiols, while the remaining is composed of low molecular weight thiols such as cysteine, cysteinglysin, glutathione (GSH), homocysteine and gamma-glutamylcysteine.[19] Oxidation of thiols and the resulting disulfide bond formation are indicators of early cellular response to OS.[13 20] During OS, thiol groups are converted to disulfide forms and these forms can reduce back to thiol groups, thereby achieving dynamic thiol balance.[17]

Table 4 Correlation analysis of laboratory parameters in patients with CLL

Variables		Leukocyte	Lymphocyte	Direct bilirubin	Total bilirubin	ALP	TOS	OSI	IMA
Native thiol (µmol/L)	r	−0.447	−0.491	0.363	0.386	0.395	−0.393	−0.325	−0.122
	P value	0.001	0.001	0.010	0.006	0.05	0.005	0.023	0.404
Total thiol (µmol/L)	r	−0.420	−0.445	0.326	0.343	0.340	−0.339	−0.266	−0.142
	P value	0.03	0.001	0.022	0.016	0.017	0.017	0.064	0.331
Disulfide (µmol/L)	r	0.143	0.265	−0.220	−0.235	−0.187	0.317	0.315	0.052
	P value	0.240	0.066	0.128	0.105	0.199	0.026	0.028	0.722
Disulfide/Native thiol (%)	r	0.597	0.380	−0.357	−0.380	−0.322	0.400	0.339	0.071
	P value	0.001	0.007	0.012	0.007	0.024	0.004	0.017	0.630
Disulfide/Total thiol (%)	r	0.651	0.389	−0.354	−0.376	−0.329	0.402	0.341	0.072
	P value	0.001	0.006	0.013	0.008	0.021	0.004	0.017	0.624
Native thiol/Total thiol (%)	r	−0.651	−0.393	0.357	0.380	0.320	0.754	0.343	−0.084
	P value	0.001	0.005	0.012	0.007	0.025	0.0001	0.016	0.566

*Pearson's correlation test.
ALP, alkaline phosphatase; IMA, ischemia-modified albumin; OSI, oxidative stress; TOS, total oxidant status.

J Investig Med: first published as 10.1136/jim-2022-002568 on 29 August 2022. Downloaded from file:/ on April 19, 2024 by guest. Protected by copyright.

Hanikoglu *et al* showed that before radical prostatectomy (RP) native thiol, total thiol and TAS levels were significantly lower in the patient group as compared with the controls. However, disulfide level was significantly higher in the patient group with respect to controls. They concluded that this decrease is due to the deterioration of the capacity to resist oxidant stress. They also reported that after RP native thiol, total thiol and TAS levels were increased while disulfide levels were decreased. They concluded that thiol-disulfide homeostasis reduced back to thiols side.[21] In another study, Eryilmaz *et al* showed that native and total thiol were decreased while disulfide level was increased in patients with breast cancer compared with the controls. They hypothesized that the change in thiol-disulfide homeostasis may be due to oxidation/reduction reactions caused by ROS.[22] In the same line with the previous studies, we found that native and total thiol levels were lower while disulfide levels higher in patients with CLL in contrast to the controls. One reason of the decrease in serum thiol levels may be the continuous depletion of sulfhydryl-containing antioxidant molecules, particularly glutathione, to remove ROS as previously suggested.[23] This further suggests that OS may play a role in etiopathogenesis of disease.

In the literature, Fidan *et al* showed that TAS levels were lower while TOS and OSI levels were higher in patients with gastric cancer compared with the controls. They concluded that low levels of antioxidant may play a role in carcinogenesis.[24] In our study, we found that TAS levels were lower while TOS and OSI levels were significantly higher in patients with CLL than controls. We concluded that increased OS may deplete antioxidants molecules to remove ROS. In addition, decreased antioxidant levels in patients with CLL may increase TOS and OSI levels due to increased OS. We also hypothesized that increased ROS in CLL and the resulting decreased antioxidant capacity may lead to carcinogenesis.

In the literature, several studies found serum IMA levels were higher in patients with gastric, prostate, neuroblastoma, and soft tissue cancer compared with the controls. They concluded that the increase in the level of IMA may be related to OS and functional decrease of the antioxidant defense system.[24–26] Our study is the first study to evaluate IMA levels in patients with CLL. In accordance with the literature, we found that serum IMA levels were higher in patients with CLL than controls. We assumed that increased IMA levels may play an important role in the production of ROS under OS.

Limitation of the study

The limitation of this study is a small sample size. Large sample size studies are needed to further evaluate the results.

CONCLUSION

In conclusion, we found increased TOS and OSI levels with decreased TAS levels in patients with CLL. These findings strongly favor the impairment of oxidant-antioxidant balance. We also found decreased levels of native and total thiol while increased disulfide in patients with CLL. This might indicate that formation of disulfide leads to decrease in antioxidant capacity in patients with CLL. Measurement of dynamic thiol-disulfide levels can contribute to follow-up of the disease as a potential biomarker particularly for the early identification of CLL and further aid to monitor the progression of the disease.

Acknowledgements The authors thank patients and controls for their voluntary support for the conduction of this study.

Contributors Conception and design of the research: HE. Acquisition of data: HE, RC, OÖ, SCT. Analysis and interpretation of data: HE, RC, OÖ. Statistical analysis: HE. Drafting the manuscript: HE. Revision of manuscript for important intellectual content: HE, RC, OÖ, SCT. Guarantor: HE.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study was approved by the Hatay Mustafa Kemal University Clinical Research Ethics Committee (protocol number: 2021/111). Written informed consent was obtained from all participants.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement No data are available.

ORCID iD

Huseyin Erdal http://orcid.org/0000-0003-0786-5077

REFERENCES

1 Cronin KA, Ries LAG, Edwards BK. The surveillance, epidemiology, and end results (SEER) program of the National Cancer Institute. *Cancer* 2014;120 Suppl 23:3755–7.
2 Hallek M, Shanafelt TD, Eichhorst B. Chronic lymphocytic leukaemia. *Lancet* 2018;391:1524–37.
3 Slager SL, Benavente Y, Blair A, *et al*. Medical history, lifestyle, family history, and occupational risk factors for chronic lymphocytic leukemia/small lymphocytic lymphoma: the InterLymph non-Hodgkin lymphoma subtypes project. *J Natl Cancer Inst Monogr* 2014;2014:41–51.
4 Liu J, Chen G, Pelicano H, *et al*. Targeting p53-deficient chronic lymphocytic leukemia cells in vitro and in vivo by ROS-mediated mechanism. *Oncotarget* 2016;7:71378–89.
5 Sies H, Berndt C, Jones DP. Oxidative stress. *Annu Rev Biochem* 2017;86:715–48.
6 Özcan O, Erdal H, Çakırca G, *et al*. Oxidative stress and its impacts on intracellular lipids, proteins and DNA. *J Clin Exp Invest* 2015;6:331–6.
7 Klaunig JE. Oxidative stress and cancer. *Curr Pharm Des* 2018;24:4771–8.
8 Zhang P, Li T, Wu X, *et al*. Oxidative stress and diabetes: antioxidative strategies. *Front Med* 2020;14:583–600.
9 Donia T, Khamis A. Management of oxidative stress and inflammation in cardiovascular diseases: mechanisms and challenges. *Environ Sci Pollut Res Int* 2021;28:34121–53.
10 Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. *J Biol Chem* 2013;288:26489–96.
11 Baba SP, Bhatnagar A. Role of thiols in oxidative stress. *Curr Opin Toxicol* 2018;7:133–9.
12 Erel O, Neselioğlu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014;47:326–32.
13 Özcan O, Erdal H, İlhan G, *et al*. Plasma Ischemia-Modified albumin levels and dynamic thiol/disulfide balance in sickle cell disease: a case-control study. *Turk J Haematol* 2018;35:265–70.
14 Dirican N, Dirican A, Sen O, *et al*. Thiol/disulfide homeostasis: a prognostic biomarker for patients with advanced non-small cell lung cancer? *Redox Rep* 2016;21:197–203.
15 Otal Y, Kahraman FA, Haydar FG, *et al*. Dynamic thiol/disulfide homeostasis as oxidative stress marker in diabetic ketoacidosis. *Turk J Med Sci* 2021;51:743–8.
16 Cakirca GCM, Erdal H, Neselioglu S, *et al*. Investigation of thiol/disulfide homeostasis in familial Mediterranean fever patients. *Clin Anal Med* 2018;9:231–4.
17 Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;38:1103–11.
18 Bar-Or D, Curtis G, Rao N, *et al*. Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. *Eur J Biochem* 2001;268:42–7.

J Investig Med: first published as 10.1136/jim-2022-002568 on 29 August 2022. Downloaded from file:/ on April 19, 2024 by guest. Protected by copyright.

19 Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Radic Biol Med* 2013;65:244–53.
20 Inayama T, Oka J, Kashiba M, *et al*. Moderate physical exercise induces the oxidation of human blood protein thiols. *Life Sci* 2002;70:2039–46.
21 Hanikoglu F, Hanikoglu A, Kucuksayan E, *et al*. Dynamic thiol/disulphide homeostasis before and after radical prostatectomy in patients with prostate cancer. *Free Radic Res* 2016;50:S79–84.
22 Eryilmaz MA, Kozanhan B, Solak I, *et al*. Thiol-disulfide homeostasis in breast cancer patients. *J Cancer Res Ther* 2019;15:1062–6.
23 Arnér ESJ, Holmgren A. The thioredoxin system in cancer. *Semin Cancer Biol* 2006;16:420–6.
24 Fidan E, Mentese A, Kavgaci H, *et al*. Increased ischemia-modified albumin levels in patients with gastric cancer. *Neoplasma* 2012;59:393–7.
25 Stachowicz-Stencel T, Synakiewicz A, Owczarzak A, *et al*. Ischemia-modified albumin as a biochemical marker in children with neuroblastoma and soft tissue sarcomas. *J Clin Lab Anal* 2011;25:255–8.
26 Mastella AK, Moresco RN, da Silva DB, *et al*. Evaluation of ischemia-modified albumin in myocardial infarction and prostatic diseases. *Biomed Pharmacother* 2009;63:762–6.

J Investig Med: first published as 10.1136/jim-2022-002568 on 29 August 2022. Downloaded from file:/ on April 19, 2024 by guest. Protected by copyright.