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Original Article

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Thiol-disulfide homeostasis in children with febrile neutropenia

Abstract

Aim: The body's antioxidant system processes rely on thiol-disulfide equilibrium to function correctly. The purpose of this study was to look into dynamic thiol/disulfide homeostasis (TDH) as an endogenous antioxidant indicator in febrile neutropenic (FN) children with acute lymphoblastic leukemia (ALL).
Materials and methods: The study included 66 acute lymphoblastic leukemia patients, including 33 with FN and 33 controls. Both groups were compared for TDH and ischemia-modified albumin (IMA). TDH parameters were assessed using a unique spectrophotometric approach disclosed earlier by Neşelioğlu and Erel.
Results: Total thiol, native thiol, disulfide, and native thiol/total thiol levels were significantly lower in patients with FN than in the controls ($p< 0.05$). Disulfide/total thiol, disulfide/native thiol, and IMA levels were significantly higher in the FN group than in controls ($p<0.05$).
Conclusions: TDH could be a simple tool that could be used in routine screening as an indicator of thiol-specific oxidative stress, particularly in pediatric febrile neutropenia.

Keywords: Thiol/disulfide homeostasis, oxidative stress, febrile neutropenia, acute lymphoblastic leukemia

INTRODUCTION

Infection is a leading reason of death in leukemia patients. Bone marrow suppression caused by radiation or chemotherapy causes leukopenia, particularly neutropenia, which increases the risk of bacterial infection. Fever is frequently the first sign of a potentially fatal infection, especially during neutropenia. Inflammation signs and symptoms may be absent in 50% of patients. In the neutropenic host, fever is often the only symptom of a hidden infection (1). However, this symptom may be absent in some infected patients who are instead hypothermic, hypotensive, listless, or disoriented. If there is any evidence of clinical deterioration in a neutropenic patient, infections must be considered and treated empirically.

Thiol is an organic molecule that contains the sulfhydryl group (-SH), which is essential in preventing oxidative stress in cells (2). The most common type of dynamic, redox responsive covalent connection formed by two thiol classes is disulfide. Dynamic thiol-disulfide homeostasis (TDH) represents the condition of thiols (-SH) and disulfides, which is the alteration of thiol oxidation in proteins (-S-S-). The dynamic thiol/disulfide

(SH/SS) equilibrium condition is essential for antioxidation, apoptosis, cell protection, signal activation, detoxifying, and enzyme-mediated regulation (2-4). TDH was mainly analyzed in the past unilaterally, but it is now analyzed bilaterally utilizing the new procedure described by Neselioglu and Erel (2,5).

This research aimed to look into the dynamic thiol/disulfide homeostasis in febrile neutropenic children with acute lymphoblastic leukemia (ALL) as an oxidative stress marker and show whether thiol hemostasis is a sign of infection in these patients who may be asymptomatic due to neutropenia.

MATERIAL AND METHODS

The research was conducted between January 2021 and July 2021 at Bursa Yüksek İhtisas Training and Research Hospital, Department of Pediatric Hematology and Oncology, Bursa, Turkey. The study comprised 66 children followed up with acute lymphoblastic leukemia (ALL). Thirty-three of them had febrile neutropenia, while the remaining 33 were controls of similar age and gender. The children in the control group had no neutropenia or infectious diseases. The research protocol was approved by the Hospital's Ethical Commission (2011-KAEK-25 2020/12-06), and signed informed consent was acquired from the patients, their families, and the controls.

The presence of fever during a neutropenia episode is referred to as febrile neutropenia. A neutrophil count (ANC) of <500 cells/mm3 is defined as neutropenia or a decrease to <500 cells/mm3 during the following 48 hours. Fever is regarded as a specific oral temperature scale of >38.3° C (101° F) and even a temperature of >38.0° C (100.4 F) maintained over a one-hour duration, according to the Infectious Diseases Society of America (IDSA). Fever is described as an oral temperature of even more than 38.5°C or two subsequent measurements of more than 38.0°C for two hours, according to the European Society of Medical Oncology (ESMO) (6,7).

Thiol (-SH) is an organic molecule made up of sulfur and hydrogen atoms that contain the sulfhydryl group. Disulfides are the most frequent narrative, redox adaptable intermolecular connection between two thiol groups (-S-S-). TDH (dynamic thiol-disulfide homeostasis) represents the levels of disulfides and thiols and is the reversal of thiol oxidation in proteins. Patients' blood was drawn, and serum was derived by centrifuge tube at 1500g for 10 minutes and then stored at 80°C without protectant till the analysis. SH/SS homeostasis parameters were assessed using a unique spectrophotometric approach disclosed earlier by Erel and Neşelioğlu (2). The goal of this novel technology was to measure such thiol and disulfide concentrations in the blood. First, without pretreatment, accessible thiol in serum/plasma was found. That was presumed to be native thiol (NT). Then, using sodium borohydride as a pretreatment, dynamic disulfide bonds were converted to free sulfhydryl groups (NaBH4). The following evaluation was taken, and the value was recognized as total thiol (TT). Additional savings of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) the produced base pairs may be reduced further during this stage. All leftover NaBH4 residues were eliminated with formaldehyde to avoid positive influence. Finally, disulfide accounted for about 50 percent of the variance among TT and NT. 2-mercaptoethanol was employed during the measurement technique to establish a linear calibration curve. They also employed two wavelengths: 415 nm for the primary and 700 nm for the supplementary wavelength.

NT in this approach represented thiol (-SH), whereas oxidized thiol (-S-S) was reflected by disulfide. Total thiol is the sum of the NT and disulfide levels. In organisms to elucidate dynamic TDH, this approach examined NT, total thiol (TT), disulfide concentration, the proportion of disulfide to total thiol, disulfide to native thiol, and native to total thiol. The results were presented in micromole per liter using an automated clinical chemistry analyzer (Cobas 501; Mannheim, Germany). After incubation with patient serum, ischemia-modified albumin (IMA) was determined using a colorimetric system described by Bar-Or et al. based on the measurement of unshackled cobalt, and the findings were presented as absorbance units (ABSU) (8). Albumin modified IMA was computed using the formula = (individual serum albumin concentration/population median albumin concentration) IMA value (2). Serum interleukin-6 (IL-6) amounts were calculated using the chemiluminescent method and Serum C-reactive protein (CRP) with a nephelometer.

IBM SPSS Statistics for Windows, Version 22.0, was used for statistical analysis (IBM Corp., Armonk, NY). The mean and standard deviation were used to express continuous variables with a normal distribution. Numbers and percentages were used to represent categorical variables. For the normal and abnormal distributions of continuous data, the student t-test and Mann-Whitney U test were used, respectively. The relationship between variable groups was assessed using variant analysis. As the statistical significance level $p < 0.05$ was used.

RESULTS

The study included 33 febrile neutropenic patients (mean age: 98.78±63.02 months) and 33 healthy controls (mean age: 97.69±51.26 months). In terms of sex and age, the patient and control groups were similar Total thiol, native thiol, disulfide, and native thiol/total thiol levels were significantly lower in the febrile neutropenia group than in controls ($p< 0.05$), as shown in Table1 and Figure 1, Figure 2, Figure 3. Disulfide/total thiol and disulfide/native thiol levels were significantly higher in the febrile neutropenia group than in controls ($p<0.05$), as shown in Table1. IMA levels were significantly higher in the febrile neutropenia group than in controls ($p<0.05$), as shown in Figure 4. There was no significant relation between serum CRP, IL-6, and SH/SS homeostasis ($p>0.05$).

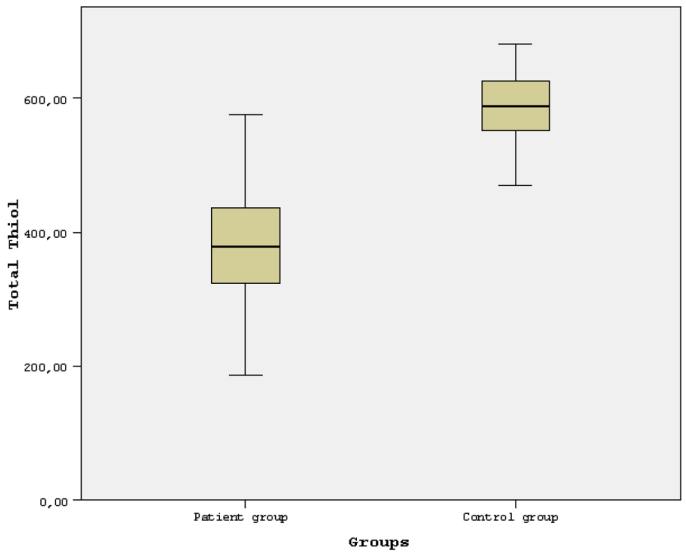

Figure 1. Total thiol levels in febrile neutropenia and the control groups

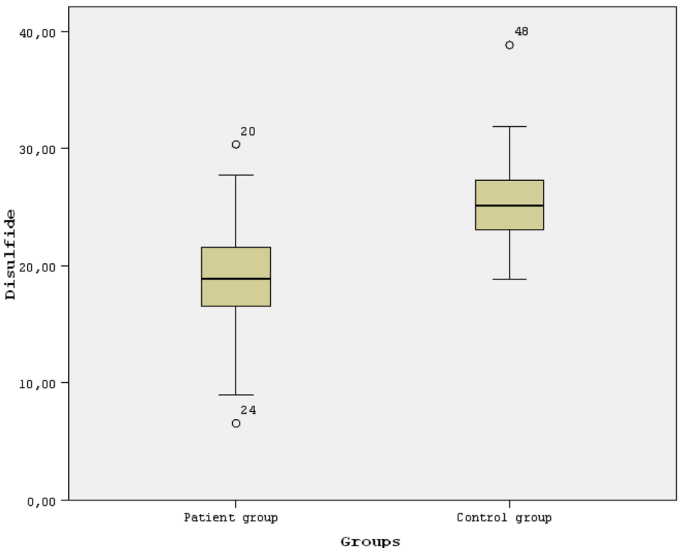

Figure 3. Disulfide levels in febrile neutropenia and the control groups

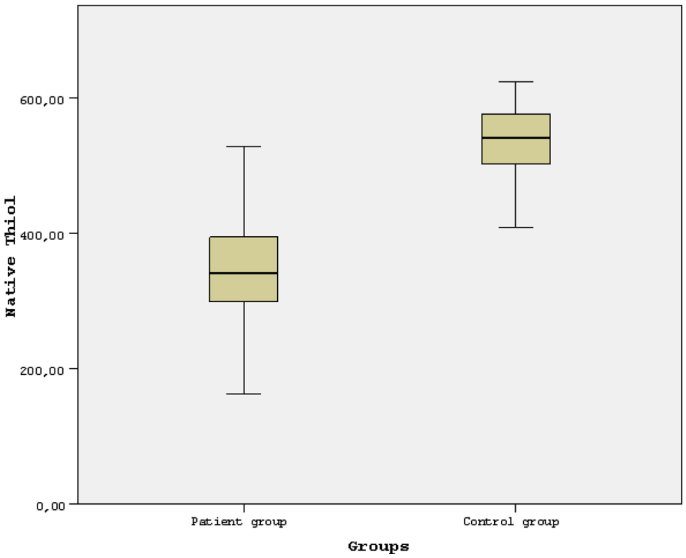

Figure 2. Native thiol levels in febrile neutropenia and the control groups

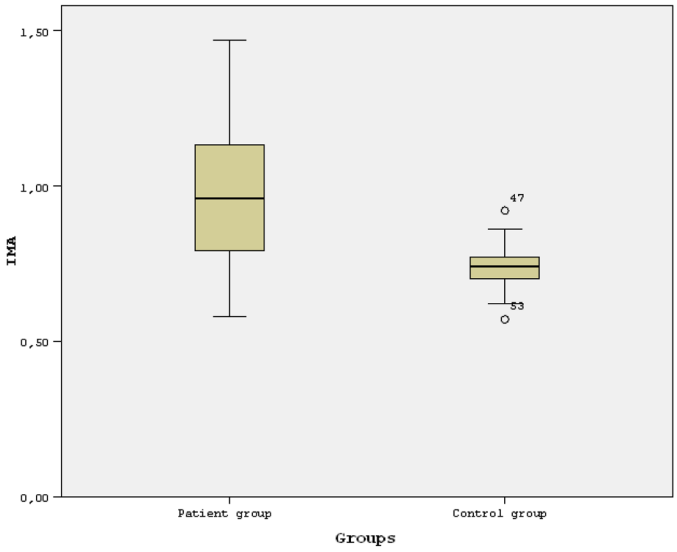

Figure 4. Ischemia-modified albumin (IMA) levels in febrile neutropenia and the control groups

Table1. Demographic characteristics and thiol/disulfide homeostasis values of the febrile neutropenia and control groups

Parameters	Febrile Neutropenia (n=33) Mean ±SD or n (%)	Controls (n=33) Mean ±SD or n(%)	*P*
Age(months)	98.78±63.02	97.69±51.26	1
Gender			
Female	8(26.4)	8(26.4)	0.939
Male	25(74.6)	25(74.6)	
Native thiol(µmol/L)	349.80±93.53	537.77±51.19	0.001
Total thiol (SH)(µmol/L)	388.11±99.71	588.98±52.50	0.001
Disulfide (SS)(µmol/L)	19.15±5.17	25.6±3.9	0.001
(Disulfide/Total thiol) *100	5.04±1.15	4.37±0.73	0.007
(Native Thiol/Total thiol) *100	89.92±2.31	91.25±1.45	0.007
Disulfide/Native thiol (%)	5.64±1.43	4.80±0.88	0.006
IMA (ABSU)	0.99±0.25	0.73±0.07	0.001

DISCUSSION

A healthy lifestyle requires a balance of oxidants and antioxidants. Thiols are necessary antioxidant buffers because they directly contact nearly all physiological oxidants. (5,9-12). Thiol protein groups represent more than half of the total plasma antioxidant capacity in healthy people. Earlier, thiols could only be measured in one direction (5). TDH is measured using a method published recently by Neşelioğlu and Erel (2). TDH including total thiol, native thiol, and disulfide levels, can now be counted easily and repeatedly using a fully automated methodology with high sensitivity and specificity.

It was discovered that oxidative stress was linked to infectious diseases such as hepatitis C, pneumonia, Brucellosis, sepsis, appendicitis, urinary tract infection, and tonsillopharyngitis (13-21). Thiol levels have recently been regarded as an antioxidant measure in various disorders. This research aimed to look into changes in SH/SS homeostasis, a new marker of oxidative stress, in pediatric febrile neutropenic patients. That is the first research to look into thiol homeostasis as an indicator of oxidative condition in children with febrile neutropenia.

Several studies have discovered a connection between oxidative status and febrile neutropenia, and infectious damage worsens the intensity of oxidative stress (22-24).

Native thiol, total thiol, and disulfide values were lower in pediatric patients with FN, as was the native thiol/total thiol ratio. Both the disulfide/native thiol and disulfide/total thiol ratios were elevated in FN patients. These findings indicate the presence of oxidative stress in FN, while the decrease in disulfide level implies that the oxidation reaction has progressed. Because thiol metabolism forms disulfide bonds, the actuality that the disulfide level in FN did not increase despite increased thiol usage suggests that sophisticated protein oxidation was present. Because of the transformation of protein to its end products due to advanced protein oxidation, the level of disulfide was found to be below.

Multiple types of research on advanced oxidation protein final products in various diseases have been conducted. These studies found that children with infectious diseases had higher disulfide/native thiol ratios and disulfide/total thiol ratios and reduced native thiol, total thiol levels, and native thiol/total thiol ratios (13-21,25,26). Because of these changes in the thiol-disulfide balance, it may be a completely new marker for determining the severity of febrile neutropenia in ALL patients.

The most abundant serum protein, human serum albumin (HSA), is responsible for 70% of the serum's scrounging capacity for free radicals (27). Under oxidative stress, IMA is formed when oxidative stress to the aminoterminal of HSA occurs within hours of reperfusion; IMA levels return to normal. Recent research has discovered elevated levels of IMA, a sensitive marker in several oxidative stress-related conditions such as diabetes, coronary artery disease, renal disease, and endocrine disorders (28-31). We also assessed the importance of IMA in FN. In our investigation, blood levels of IMA were considerably more significant in FN patients than in controls, and it is consistent with the findings of other studies (2,32-34). We discovered that, like TDH, the IMA could be used as an oxidative stress indicator.

CONCLUSION

As a result, we discovered that in FN, TDH favors oxidative stress. This novel technique could be a simple tool for screening tests as a marker of thiol-specific oxidative stress, especially in children with FN. The limited number of patients and the lack of post-treatment values are the limitations of our study, whereas this is the first study that we are aware of that looks at TDH equilibrium in this group of patients.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
The research protocol was approved by the Hospital's Ethical Commission (2011-KAEK-25 2020/12-06), and signed informed consent was acquired from the patients, their families, and the controls.

REFERENCES

1. Kebudi R, Kizilocak H. Febrile neutropenia in children with cancer: approach to diagnosis and treatment. Curr Pediatr Rev. 2018;14:204-9.
2. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47:326-32.
3. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochemical Pharmacology. 2006;71: 551-64.
4. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems and apoptosis. Free Radical Biology and Medicine. 2010;48:749-62.
5. .Ellman G, Lysko H. A precise method for the determination of whole blood and plasma sulfhydryl groups. Analytical Biochemistry. 1979; 93:98-102.
6. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases society of america. Clin Infect Dis. 2011;52:e56-e93.
7. de Naurois J, Novitzky-Basso I, Gill MJ, et al. Management of febrile neutropenia: ESMO Clinical Practice Guidelines. Ann Oncol. 2010;21Suppl 5:v252-v6.
8. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med. 2000;19:311-5.

9. Ergin M, Cendek BD, Neşelioğlu S, et al. Dynamic thiol-disulfide homeostasis in hyperemesis gravidarum. J Perinatol. 2015;35:788-92.

10. Otal Y, Demircan S, Şener A, et al. Acute renal failure and thiol-disulfide homeostasis. Journal of Nephrology Therapeutic. 2018;8:312-2.

11. Erel Ö, Erdoğan S. Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical aspects. Turk J Med Sci. 2020;50:1728-38.

12. Ercan Haydar FG, Otal Y, Şener A, et al. The thiol-disulphide homeostasis in patients with acute pancreatitis and its relation with other blood parameters. TJTES. 2020;26:37-42.

13. Damar Çakırca T, Ceylan MR, Koyuncu İ, Çakırca G. Thiol-disulphide balance and total oxidant-antioxidant status in patients with chronic hepatitis C. Int J Clin Pract. 2021;75:e13988.

14. Parlak ES, Alisik M, Hezer H, et al. Evaluation of dynamic thiol/disulfide redox state in community-acquired pneumonia. Saudi Med J. 2018;39:495-9.

15. Kolgelier S, Ergin M, Demir LS, et al. Impaired thiol-disulfide balance in acute brucellosis. Jpn J Infect Dis. 2017;70:258-62.

16. Temel MT, Demiryürek S, Temel L, et al. Dynamic thiol/disulfide homeostasis in children with community-acquired pneumonia. Pediatr Int. 2019;61:252-7.

17. Elmas B, Yildiz T, Yazar H, et al. New oxidative stress markers useful in the diagnosis of acute appendicitis in children: thiol/disulfide homeostasis and the asymmetric dimethylarginine level. Pediatr Emerg Care. 2020;36:362-7.

18. Aydogan S, Akduman H, Dilli D et al. The role of thiol-disulfide homeostasis in neonatal sepsis. J Matern Fetal Neonatal Med. 2021;34:1522-28.

19. Öktem A, Zenciroğlu A, Dilli D, et al. Thiol-disulfide homeostasis in neonatal patients with urinary tract infection. Am J Perinatol. [published online ahead of print, 2021 Jan 17]

20. Cakir E, Gok G, Erel O, Turan IO. Thiol/disulfide homeostasis as an early biomarker to differentiate sepsis from pneumonia in intensive care units. Comb Chem High Throughput Screen. 2021;24:1446-52.

21. Kara SS, Erel O, Demirdag TB, et al. Alteration of thiol-disulphide homeostasis in acute tonsillopharyngitis. Redox Rep. 2017;22:205-9.

22. Carr AC, Spencer E, Das A, et al. Patients undergoing myeloablative chemotherapy and hematopoietic stem cell transplantation exhibit depleted vitamin c status in association with febrile neutropenia. Nutrients. 2020;12:1879.

23. Radhakrishnan N, Dinand V, Rao S, et al. Antioxidant levels at diagnosis in childhood acute lymphoblastic leukemia. Indian J Pediatr. 2013;80:292-6.

24. Kari EJM, Kuusisto MEL, Böhm J, et al. Peroxiredoxin 6 Serum Levels and Risk of Neutropenic Infections in Diffuse Large B-cell Lymphoma. Anticancer Res. 2019;39:4925-31.

25. Kumar S, Gupta E, Kaushik S, et al. Evaluation of oxidative stress and antioxidant status: Correlation with the severity of sepsis. Scand J Immunol. 2018;87:e12653.

26. Lorente L, Martín MM, Almeida T, et al. Association between serum total antioxidant capacity and mortality in severe septic patients. J Crit Care. 2015;30:217.e7-12.

27. Bourdon E, Loreau N, Lagrost L, Blache D. Differential effects of cysteine and methionine residues in the antioxidant activity of human serum albumin. Free Radic Res. 2005;39:15-20.

28. Seshadri Reddy V, Sethi S, Gupta N et al. Significance of ischemia-modified albumin as a simple measure of oxidative stress and its discriminatory ability in diabetic retinopathy: literature review and meta-analysis. Retina. 2016;36:1049-57.

29. Voudris KV, Chanin J, Feldman DN, Charitakis K. Novel inflammatory biomarkers in coronary artery disease: potential therapeutic approaches. Curr Med Chem. 2015;22:2680-89.

30. Kotani K, Kimura S, Gugliucci A. Paraoxonase-1 and ischemia-modified albumin in patients with end-stage renal disease. J Physiol Biochem. 2011;67:437-41.

31. Erem C, Suleyman AK, Civan N et al. Ischemia-modified albümin and malondialdehyde levels in patients with overt and subclinical hyperthyroidism: effects of treatment on oxidative stress. Endocr J. 2015;62:493-501.

32. Awadallah SM, Atoum MF, Nimer NA, Saleh SA. Ischemia modified albumin: an oxidative stress marker in β-thalassemia major. Clin Chim Acta. 2012;413:907-10.

33. Yazıcı MU, Ayar G, Savas-Erdeve S, et al. Role of ischemia modified albumin serum levels as an oxidative stress marker in children with diabetic ketoacidosis. Comb Chem High Throughput Screen. 2019;22:577-81.

34. Keshavarzi F, Rastegar M, Vessal M, et al. Serum ischemia modified albumin is a possible new marker of oxidative stress in phenylketonuria. Metab Brain Dis. 2018;33:675-80.