Annals of Clinical and Analytical Medicine
Original Research

Thiol/disulfide homeostasis as a new oxidative stress marker in patients with neonatal transient tachypnea

Huseyin Erdal[1], Mehmet Semih Demirtas[2], Sibel Cigdem Tuncer[3], Oguzhan Ozcan[4]
[1] Department of Medical Genetics, Faculty of Medicine, Aksaray University, Aksaray
[2] Department of Pediatrics, Aksaray Training and Research Hospital, Aksaray
[3] Deparment of Biochemistry, Faculty of Medicine, Aksaray University, Aksaray
[4] Department of Biochemistry, Faculty of Medicine, Hatay Mustafa Kemal University, Hatay, Turkey

This study was presented in International Congress on Innovative Approaches in Medical and Health Sciences, Online, Turkey, 2022

Abstract

Aim: Transient tachypnea of the newborn (TTN) is the most common cause of respiratory distress occurring in delayed clearance of lung fluid. This is the first report to examine thiol-disulfide homeostasis in patients with TTN.

Material and Methods: Thirty TTN and 30 controls were included in the present study. The dynamic thiol-disulfide balance was determined by the new colorimetric method developed by Erel et al. TAS, TOS and OSI levels were evaluated using the previously described method developed by Erel.

Results: Thiol levels were found to be significantly lower between patient and the control groups. However, disulfide levels were not significantly higher in the TTN group compared with the control groups. Disulfide/native thiol, disulfide/total thiol and native/total thiol levels were statistically significantly different between the TTN and control groups. Moreover, we found that TAS, TOS and OSI levels were also statistically significantly different between patient and control groups.

Discussion: This study indicates that the measurement of dynamic thiol-disulfide homeostasis may contribute to the pathophysiological mechanism, and follow-up of the disease in patients with TTN. In addition, increased TOS and decreased TAS levels may be related with increased oxidative stress and a functional reduction of antioxidant defense system

Keywords

Transient Tachypnea, Oxidative Stress, Thiol/Disulfide Homeostasis, Newborn, Respiratory Distress

DOI: 10.4328/ACAM.21457 Received: 2022-10-19 Accepted: 2022-11-22 Published Online: 2022-12-07 Printed: 2023-03-01 Ann Clin Anal Med 2023;14(3):208-211
Corresponding Author: Huseyin Erdal, Department of Medical Genetics, Faculty of Medicine, Aksaray University, Aksaray, Turkey.
E-mail: herdalyfa@gmail.com P: +90 543 414 08 15
Corresponding Author ORCID ID: https://orcid.org/0000-0003-0786-5077
This study was approved by the Clinical Research Ethics Committee of Hatay Mustafa Kemal University (Date: 2020-10-22 , No: 2020/08)

Introduction

The most common reason for hospitalization of an infant to the neonatal intensive care unit is respiratory distress (RD). RD in the newborn is considered one or more signs of increased work of breathing, such as tachypnea, nasal enlargement, chest tightness, or grunting [1]. Transient tachypnea of the newborn (TTN) is a common cause of respiratory distress in neonates and results from impaired fetal lung fluid clearance [2]. During fetal life, fluid is secreted into the alveoli to maintain normal growth and development, and fetal lung volume approaches the functional residual capacity that would be created when air breathing is initiated [3]. Fetal alveolar fluid filling the alveoli during the intrauterine period has a positive effect on lung mechanics by stretching the lungs. In order to have effective gas exchange after birth, it is necessary to clean the existing liquid from the environment [4]. TTN was first described in 1966 and is the most common cause of RD in term and late preterm infants [2]. TTN is usually characterized by a respiratory rate of over 60 breaths per minute and symptoms of respiratory distress [5]. The estimated incidence of TTN is 4.0-5.7 per 1000 term births [2,6]. Common risk factors in TTN formation include precipitous delivery, fetal distress, maternal sedation, low birth weight and gestational diabetes [7]. Although TTN is considered a benign condition in infants, it is known to increase respiratory and stress load [8]. Oxidative stress (OS) occurs as a result of the deterioration of the balance between free radicals and antioxidants and is responsible for the pathogenesis of various types of diseases such as cancer, neurological and inflammatory disorders [9-12]. Newborn infants are very sensitive to oxidative damage due to their limited antioxidant capacity [13]. Therefore, the evaluation of OS levels in newborns plays a very vital role in terms of preventing unfavorable consequences that may occur due to OS and applying appropriate treatment methods. Thiol groups are the primary target of reactive oxygen species (ROS) produced in the cell as a result of OS. Thiols are compounds that contain a sulfhydryl functional group in their structure. Thiols are exposed to oxidation reactions by increased oxidant molecules as a result of OS and disulfide structures are formed [14]. Disulfide bonds are reduced back to thiol groups by reducing agents, and thus a dynamic thiol-disulfide homeostasis is achieved. To the best of our knowledge, this is the first report to evaluate dynamic thiol-disulfide levels in patients with TTN.

Material and Methods

Study Design

This study was performed at the 3rd level neonatal intensive care center of Aksaray University Training and Research Hospital Neonatal Intensive Care Unit, in order to determine the thiol-disulfide balance in patients with TTN.

Patient and Control Groups

A total of 60 subjects, including 30 controls and 30 TTN, were included in this study. There was no statistically significant difference in terms of age and gender in the patient group and healthy controls. Demographic data of the study and control groups were collected from the hospital automation system. Infants born 37 weeks before or after 39 weeks, those with congenital pneumonia and RDS, those with persistent hypoglycemia, hypocalcemia and polycythemia, and infants with heart disease or meconium aspiration were extricated from the current study. Our study was conducted in accordance with the ethical standards of the World Medical Association Helsinki Declaration Principles. Written informed consent was obtained from all patients and control families who agreed to participate in the study. The present study was approved by the Hatay Mustafa Kemal University Clinical Research Ethics Committee (protocol number: 2020/08).

Sample Collection of the Study

All working samples were taken into ethylenediaminetetraacetic acid (EDTA) vacuum tubes and centrifuged at 3600 x rpm for 10 minutes. The serum obtained was aliquoted into Eppendorf tubes and kept in a deep freezer at -80°C until analysis.

Measurement of TAS and TOS levels

Total oxidant status (TOS) and total antioxidant status (TAS) levels were measured spectrophotometrically based on the method developed by Erel [15]. The measured data were expressed in μmol Trolox equivalent per liter for TAS and mmol H2O2 equivalent per liter for TOS. Then, oxidative stress index (OSI) (OSI (arbitrary unit) =TOS (μmol H2O2 Eq/L)/TAS (μmol Trolox Eq/L) ×100) was calculated.

Determination of Thiol Levels

Thiol levels (native and total) were determined by the fully automatic spectrophotometric method previously described by Erel and Neselioglu [16]. Concisely, disulfide bonds are reduced to thiol groups via sodium borohydride (NaBH4). In order to prevent reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic) acid), the remaining NaBH4 was consumed and removed with formaldehyde from the environment. Disulfide levels were determined by dividing the difference obtained by subtracting native thiols from total thiols by two.

Statistical Analysis

IBM SPSS statistics package program version 22 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The normal distribution of the data was determined by the Shapiro-Wilk test. The mean differences between the two independent groups were compared with Student's t-test. A comparison of values that did not fit the normal distribution was carried out using the Mann-Whitney U test. The Spearman correlation test was used for correlation analysis. The P-value < 0.05 was accepted as the level of statistical significance.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

The present study consisted of 60 patients, 30 in the TTN group and 30 in the control group. There was no statistically significant difference between the study groups in terms of age and gender (Table 1).

In the TTN group, 14 (46%) of the patients were male and 16 (54%) were female. In the control group, 16 (54%) of the participants were male and 14 (46%) were female. The birth weight was 3339.6 (2850-3860) g. in the control group, it was 3160 (2650-3800) g. in the patient group (p<0.034). There was no statistically significant difference in laboratory parameters between the two groups (Table 2).

Native and total thiol levels were found to be significantly

Table 1. Demographic features of participants.

Parameter		TTN (n=30) n %		Control (n=30) n %		p
Gender	Male	14 (46%)		16 (54%)		0.71*
	Female	16 (54%)		14 (46%)		
Gestational age (week)		38.6±1.3		39.3±0.65		0.47¥
Mode of delivery CS/VD		C/S	15 (%50)	C/S	24 (%80)	0.18*
		VD	15 (%50)	VD	6 (%20)	

*: Chi-Square test, ¥: Student t –test C/S: cesarean section, VD: vaginal delivery

Table 2. Laboratory parameters of study and control groups.

Parameter	TTN (n=30) Mean (min-max)	Control (n=30) Mean (min-max)	p value§
Birth Weight (g)	3160 (2650-3800)	3339.6 (2850-3860)	0.034
Gestational age (week)	38.6 (36- 40)	39.3 (38-40)	0.056
Sodium (mmol/L)	140 (136.5-149)	145 (135-167)	0.20
Potassium (mmol/L)	4.9 (3.4 - 6.4)	3.8 (3.1-5.3)	0.165
Calcium (mg/dL)	7.40 (5.6-11.0)	7.72 (6.1-11.0)	0.329
Glucose (mg/dL)	96.0 (65- 120)	95.5 (65-120)	0.853
CRP (mg/L)	2.04 (0.14-4.6)	0.96 (0.14-1.8)	0.012
ALT (U/L)	33.5 (24-46)	34.6 (24-46)	0.464
AST (U/L)	34.2 (24-47)	30.4 (23-38)	0.017
Albumin (g/dL)	4.5 (3.3-4.9)	4.4 (3.6-5.1)	0.201
Ferritin (ng/mL)	153.3 (93-196)	141.4 (93-176)	0.046

CRP: C-reactive protein, ALT: Alanine aminotransferase, AST: Aspartate transaminase, §Man-Whitney U test

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

Parameter	TTN (n=30) Mean (min-max)	Control (n=30) Mean (min-max)	p value§
Total thiol (µmol/L)	468.2 (339-648)	501.2 (438-648)	0.008
Native thiol (µmol/L)	426.6 (309-604)	466.6 (396-604)	0.001
Disulfide (µmol/L)	20.8 (10-35)	17.3 (10-27)	0.064
Disulfide / Native thiol	4.9 (2.5-9.3)	3.7 (1.9-5.9)	0.005
Disulfide /Total thiol	4.4 (2.4-7.9)	3.5 (1.8-5.3)	0.005
Native thiol /Total thiol	91.0 (84.2-95.2)	93.0 (89.3-96.2)	0.005
TAS (nmol Trolox/L)	1.6 (0.68-3.45)	1.9 (1.3-3.5)	0.006
TOS (µmolH2 O2 Equiv./L)	15.0 (6.9-32.1)	9.3 (4.8-23.4)	0.001
OSI (AU)	1.0 (0.30-2.1)	0.50 (0.26-1.13)	0.001

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index §: Man-Whitney U test

lower in patients with TTN compared with the control subjects (p<0.001). However, disulfide levels were not significantly higher in the TTN subjects compared with the control group (p<0.064). Disulfide/native, disulfide/total and native/total levels were statistically significantly different between the TTN and control groups (p<0.005). Moreover, TAS, TOS and OSI levels were also statistically significantly different between patient and control groups (p<0.001) (Table 3).

Discussion

We demonstrated that total and native thiol levels were significantly lower in patients with TTN than in control subjects. However, disulfide levels were significantly higher in patients with TTN compared to the control subjects. We also demonstrated that serum TAS levels were lower in patients with TTN group than in healthy controls. However, TOS and OSI levels were significantly higher in the patient group compared to 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 report investigating thiol-disulfide homeostasis as an oxidative marker in patients with TTN.

The excess need for oxygen immediately after birth leads to an increase in reactive oxygen species and a decrease in antioxidants. OS occurs as a result of the disruption of the balance between free radicals and antioxidants and is responsible for the pathogenesis of several neonatal diseases such as respiratory distress syndrome, bronchopulmonary dysplasia, and retinopathy of prematurity [17-19]. Thiols are organic compounds containing a sulfhydryl group in their structure and are very sensitive to oxidation reactions [20]. After oxidation reactions, thiol groups convert into disulfide bonds as a result of their interaction with free radicals. After that, they are reduced back to thiol groups by antioxidants and thus a dynamic thiol-disulfide balance is achieved.

In the study of Unal et al. in low-birth-weight preterms, they showed that both thiols and disulfide values were high and disulfide values were decreased in the first week measurements, but thiols increased compared to the first week as a result of the third-week measurement [21]. They concluded that since breast milk contains a high amount of glutathione, the serum glutathione levels of breastfed preterms may have increased and this may cause an augmentation in thiol levels. They also reported that increased disulfide levels are due to the increase in oxidative damage by phototherapy and antibiotics, which are common treatments in the first week of preterm infants.

In another study by Oktem et al. they applied antibiotherapy to newborn patients with urinary tract infections. They indicated that native and total thiol levels of the pretreatment group were lower compared to both post-treatment and control groups [22]. However, they showed that disulfide levels were higher in the pre-treatment group. They concluded that the increase in proinflammatory cytokines due to the increase in infection may cause an increase in ROS, resulting in a decrease in native and total thiol levels and an increase in disulfide levels. In the study by Aydogan et al. it was indicated that native and total thiol levels were lower in newborns with neonatal sepsis compare to controls [23]. They hypothesize that this was due to the increase in OS. In the same line with the previous studies, we found that native and total thiol levels were lower, while disulfide levels higher in the TTN group with respect to the control subjects. One of the reasons for the decrease in serum thiol levels may be the depletion of sulfhydryl-containing antioxidant molecules, especially glutathione, to remove ROS as previously suggested [24]. In another study, Bulut et al. demonstrated that TOS and TAS levels were significantly higher in newborn infants with hyperbilirubinemia compared to the controls [25]. However, the OSI level did not change significantly in the patient group compared to the control group. They hypothesized that TAS levels were higher due to the increased bilirubin levels. They also concluded that increase in TOS levels may be associated with elevated OS levels due to increased bilirubin levels. In

the present study, we found that TOS and OSI levels were higher in patients with TTN than controls. However, TAS levels were lower in patients with TTN compare to the controls. We concluded that increased OS may be related to a decrease in the antioxidant defense system. The limitation of the present study is the small sample size. Studies with larger sample size are needed for further prospective studies.

Conclusion

In conclusion, we found that TOS and OSI levels were high, while TAS levels were low in patients with TTN. These findings show us that the oxidant- antioxidant balance is impaired. We also found decreased native and total thiol levels and increased disulfide in patients with TTN groups. This result might indicate that increased OS lead to formation of disulfide bonds resulting in decreased antioxidant capacity in patients with TTN. Measurement of dynamic thiol-disulfide levels can contribute to follow-up of the disease and the application of appropriate treatment.

Limitations of the study

Our study was conducted in a single center due to sample conditions and patient population. Therefore, the sample size of our study is small. Large sample size studies are needed to evaluate the results.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *This research was supported by Aksaray University Coordination Office of Scientific Research Projects (Project # 2021-019).*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Reuter S, Moser C, Baack M. Respiratory distress in the newborn. Pediatrics in review. Pediatr Rev. 2014; 35(10): 417-28.
2. Alhassen Z, Vali P, Guglani L, Lakshminrusimha S, Ryan RM. Recent Advances in Pathophysiology and Management of Transient Tachypnea of Newborn. J Perinatol. 2021; 41(1): 6-16.
3. Guglani L, Lakshminrusimha S, Ryan RM. Transient tachypnea of the newborn. Pediatr Rev. 2008; 29(11): 59-65.
4. Katsumata M, Fujisawa T, Kamiya Y, Tanaka Y, Kamiya C, Inoue Y. Effects of long-acting muscarinic antagonists on promoting ciliary function in airway epithelium. BMC Pulm Med. 2022; 22(1):186.
5. Moresco L, Romantsik O, Calevo MG, Bruschettini M. Non-invasive respiratory support for the management of transient tachypnea of the newborn. Cochrane Database Syst Rev. 2020; 4(4): CD013231.
6. Gutvirtz G, Wainstock T, Sheiner E, Pariente G. Prematurity and long-term respiratory morbidity-what is the critical gestational age threshold? J Clin Med. 2022; 11(3):751.
7. Edwards MO, Kotecha SJ, Kotecha S. Respiratory distress of the term newborn infant. Paediatr Respir Rev. 2013; 14(1): 29-36.
8. Liem JJ, Huq SI, Ekuma O, Becker BA, Kozyrskyj LA. Transient tachypnea of the newborn may be an early clinical manifestation of wheezing symptoms. J Pediatr. 2007; 151(1): 29-33.
9. Özcan O, Erdal H, Çakırca G, Yönden Z. Oxidative stress and its impacts on intracellular lipids, proteins and DNA. J Clin Exp Invest. 2015; 6(3):331-6.
10. Cakirca G, Celik MM, Erdal H, Neselioglu S, Erel O, Basarali MK et al. Investigation of thiol/disulfide homeostasis in familial mediterranean fever patients. Clin Anal Med. 2018; 9(3): 231-4.
11. Sezgin B, Kinci MF, Pirincci F, Camuzoglu A, Erel O, Neselioglu S, et al. Thiol-disulfide status of patients with cervical cancer. J Obstet Gynaecol Res. 2020; 46(11): 2423-9.
12. Erdal H, Ciftciler R, Tuncer SC, Ozcan O. Evaluation of dynamic thiol-disulfide homeostasis and ischemia-modified albumin levels in patients with chronic lymphocytic leukemia. J Investig Med. 2022; DOI: 10.1136/jim-2022-002568.
13. Saugstad OD. Update on oxygen radical disease in neonatology. Curr Opin Obstet Gynecol. 2001; 13(2): 147-53.
14. Ozcan O, Erdal H, Ilhan G, Demir D, Gurpinar AB, Neselioglu S, 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(4): 265-70.
15. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38(12): 1103-11.
16. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014; 47(18): 326-32.
17. Hamid ERA, Ali WH, Azmy A, Ahmed HH, Sherif SH, Saleh TM. Oxidative Stress and Anti-Oxidant Markers in Premature Infants with Respiratory Distress Syndrome. Open Access Maced J Med Sci. 2019; 7(17): 2858-63.
18. Thebaud B, Goss KN, Laughon M, Whitsett AJ, Abman HS, Steinhorn HR, et al. Bronchopulmonary dysplasia. Nat Rev Dis Primers. 2019; 5(1):78.
19. Kim SJ, Port AD, Swan R, Campbell PJ, Chan PRV, Chiang FM. Retinopathy of prematurity: a review of risk factors and their clinical significance. Surv Ophthalmol. 2018; 63(5): 618-37.
20. Erel O, Erdogan S. Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical aspects. Turk J Med Sci. 2020; 50:1728-38.
21. Unal S, Isik DU, Bas AY, Erol S, Arifoglu I, Alisik M, et al. Evaluation of dynamic thiol-disulfide homeostasis in very low-birth-weighted preterms. J Matern Fetal Neonatal Med. 2019; 32(7): 1111-6.
22. Oktem A, Zenciroglu A, Dilli D, Bidev D, Özyazıcı A, Özçelik E, et al. Thiol-Disulfide Homeostasis in Neonatal Patients with Urinary Tract Infection. Am J Perinatol. 2022; 39(13): 1460-64.
23. Aydogan S, Akduman H, Dilli D, Koyuncu E, Çitli R, Erel Ö, et al. The role of thiol-disulfide homeostasis in neonatal sepsis. J Matern Fetal Neonatal Med. 2021; 34(10): 1522-8.
24. Arner ES, Holmgren A. The thioredoxin system in cancer. Semin Cancer Biol. 2006; 16 (6): 420-6.
25. Bulut O, Erek A, Duruyen S. Effects of hyperbilirubinemia on markers of genotoxicity and total oxidant and antioxidant status in newborns. Drug Chem Toxicol. 2022; 45(1): 451-5.

How to cite this article:
Huseyin Erdal, Mehmet Semih Demirtas, Sibel Cigdem Tuncer, Oguzhan Ozcan. Thiol/disulfide homeostasis as a new oxidative stress marker in patients with neonatal transient tachypnea. Ann Clin Anal Med 2023;14(3):208-211

This study was approved by the Clinical Research Ethics Committee of Hatay Mustafa Kemal University (Date: 2020-10-22, No: 2020/08)