Evaluation of Systemic Thiol/Disulfide Homeostasis as a Novel Tool for the Assessment of Oxidative Stress in Patients with Retinopathy of Prematurity

Prematüre Retinopatisi Hastalarında Oksidatif Stresin Değerlendirilmesinde Sistemik Tiyol/Disülfit Homeostazının Yeni Bir Araç Olarak İncelenmesi

Serkan AKKAYA[1], Bekir KÜÇÜK[1], Ender SIRAKAYA[1], Hatice AKKAYA[2], Çiğdem KARAKÜKCÜ[3], Mustafa Ali AKIN[4], Gamze GÖK[5], Özcan EREL[6], Zuhal TAĞ[7]

ABSTRACT

Purpose: To investigate systemic oxidative stress by evaluating serum dynamic thiol/disulfide concentrations using a novel automated homeostasis assay in retinopathy of prematurity (ROP) patients.

Materials and methods: Patients (n = 71) with a birthweight less than 1,500 g or gestational age less than 32 weeks were recruited. Groups were categorized based on those who developed ROP (n=48) and those without ROP (controls) (n = 23). The native and total thiol, disulfide levels as well as disulfide-to-native, disulfide-to-total thiol, and native thiol-to-total thiol ratios were examined and analyzed between the ROP and control groups using a novel automatized spectrophotometric assay.

Results: Levels of disulfide as well as the ratios of disulfide/native thiol and disulfide/total thiol were augmented, while the native/total thiols were reduced significantly in the ROP patients when compared to controls (mean ± SD, 19.6 ± 7.4 vs. 14.4 ± 3.4 μmol/L, $p < 0.001$ for disulfide, median (IQR), 5.8 (3.8–8.1) vs. 4.3 (3.7–5.8) μmol/L, $p = 0.017$ for disulfide/native thiol, median (IQR), 4.7 (3.3–6.1) vs. 3.7 (3.2–4.7) μmol/L, $p = 0.017$ for disulfide/total thiol, median (IQR), 80.9 (75.3–86.5) vs. 85.1 (81.0–87.1) μmol/L, $p = 0.017$ for native thiol/total thiol levels). There were no significant differences between stages of ROP and any of the thiol-disulfide parameters ($p > 0.05$ for all values).

Conclusion: These findings showed that the thiol/disulfide homeostasis was altered in the ROP patients. This metabolic imbalance may be important in the etiology of ROP.

Key words: Native thiol, oxidative stress, retinopathy of prematurity, thiol/disulfide homeostasis, total thiol.

ÖZ

Amaç: Prematüre retinopatisi (ROP) hastalarında yeni bir otomatik homeostaz analizi kullanımı ile serum dinamik tiyol/disülfit konsantrasyonlarını değerlendirerek sistemik oksidatif stresi araştırmak.

Gereç ve yöntemler: Doğum ağırlığı 1.500 gramdan düşük veya gebelik haftası 32 haftadan küçük olan hastalar (n = 71) toplandı. Gruplar, ROP gelişenlere (n = 48) ve ROP olmayanlara (kontroller) (n = 23) göre kategorize edildi. ROP ve kontrol grupları arasında yeni bir otomatikleştirilmiş spektrofotometrik analiz kullanılarak natif tiyol, total tiyol ve disülfit seviyeleri ile birlikte disülfit/natif tiyol, disülfit/total tiyol ve natif/total tiyol oranları incelendi ve analiz edildi.

Bulgular: ROP hastalarında, kontrol grubu ile karşılaştırıldığında disülfit seviyeleri ile birlikte disülfit/natif tiyol ve disülfit/total tiyol oranları istatistiksel olarak anlamlı oranda artmış olup natif/total tiyol oranları anlamlı olarak azaldı (disülfit için, ortalama ± SD, 19.6 ± 7.4 vs 14.4 ± 3.4 μmol/l, $p < 0.001$, disülfit/natif tiyol için, medyan (IQR), 5.8 (3.8–8.1) vs 4.3 (3.7–5.8) μmol/l, $p = 0.017$, disülfit/total tiyol için, medyan (IQR), 4.7 (3.3–6.1) vs 3.7 (3.2–4.7) μmol/l, $p = 0.017$, natif/total tiyol için, medyan (IQR), 80.9 (75.3–86.5) vs 85.1 (81.0–87.1) μmol/l, $p = 0.017$). ROP evreleri ile herhangi bir tiyol/disülfit parametresi arasında anlamlı fark bulunmadı (tüm değerler için $p > 0.05$).

Sonuç: Bu bulgular ROP hastalarında tiyol/disülfit homeostazının değiştiğini göstermektedir. Bu metabolik imbalans ROP etiyolojisinde önemli olabilir.

Anahtar kelimeler: Natif tiyol, oksidatif stres, prematüre retinopatisi, tiyol/disülfit homeostazı, total tiyol.

1- Uz. Dr., Kayseri Training and Research Hospital, Department of Ophthalmology, Kayseri, Türkiye
2- Uz. Dr., Zekai Tahir Burak Women's Health Training and Research Hospital, Department of Obstetrics and Gynecology, Ankara, Türkiye
3- Doç. Dr., Kayseri Training and Research Hospital, Department of Clinical Biochemistry, Kayseri, Türkiye
4- Doç. Dr., Kayseri Training and Research Hospital, Division of Neonatology, Kayseri, Türkiye
5- Asist. Dr., Faculty of Medicine, Yıldırım Beyazıt University, Department of Clinical Biochemistry, Ankara, Türkiye
6- Prof. Dr., Faculty of Medicine, Yıldırım Beyazıt University, Department of Clinical Biochemistry, Ankara, Türkiye
7- Uz. Dr., Kayseri Training and Research Hospital, Department of Pediatrics, Kayseri, Türkiye

Geliş Tarihi - Received: 25.05.2019
Kabul Tarihi - Accepted: 08.07.2019
Ret-Vit 2019; 28: 400-405

Yazışma Adresi / Correspondence Adress:
Serkan AKKAYA
Kayseri Training and Research Hospital, Department of Ophthalmology, Kayseri, Türkiye

Phone: +90 505 210 6736
E-mail: drsakkaya@gmail.com

INTRODUCTION

Retinopathy of prematurity (ROP) was first termed by Terry[1] as retrolental fibroplasia in 1942. Although much has changed in knowledge of ROP, it is still the most prominent cause of worldwide childhood blindness.[2]

In order to understand ROP pathophysiology, it is important to know the signaling events that are involved and result in altered angiogenesis in ROP. There are multiple stress factors that are important in ROP, including oxidative stress (OS), altered oxygenation, nutritional complications, inflammation, and oxidative/hypoxic pathways.[3] It is well documented that ROP patients are susceptible to OS in the phospholipid-rich retina, which is sensitive to lipid peroxidation[4] and oxygen consumption.[5] Oxygen fluctuations[6] and reactive oxygen species, produced by nicotinamide adenine dinucleotide phosphate oxidase (NADPH) oxidase,[7] can also activate vascular endothelial growth factor receptor 2 signaling and activate pathologic angiogenesis.

The imbalance happens due to OS is a dynamic mix of disulfides, protein, and low molecular weight thiols.[8] Conventional measures for analyzing these disulfides/thiols were initiated in 1979; however, Erel and Neselioglu[9] developed a colorimetric method to measure the ratio of both thiols/disulfides simultaneously.

Thiol/disulfide homeostasis is compromised in many metabolic disorders and has a role in antioxidant protection, detoxification, signal transduction, and many other cellular functions.[10,11]

The goal of this study was to analyze thiol/disulfide levels in ROP patients and premature infants without ROP. To our knowledge, this study is the first evaluating the thiol/disulfide balance in ROP using the colorimetric method developed by Erel and Neselioglu.[9]

MATERIALS AND METHODS

We enrolled 71 premature babies that were admitted to our neonatology unit between July 2016 and June 2017. The study protocol was written in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Erciyes University in Turkey. The parents or guardians of the premature babies granted their written informed consents.

The inclusion criterion was being less than 32 weeks old or having a birth weight less than 1,500 grams. Infants who had major congenital anomaly, perinatal asphyxia, twin-to-twin transfusion, and intraventricular hemorrhage greater than or equal to Grade 2 or sepsis and those receiving blood transfusion or inotrope therapy and/or developed bronchopulmonary dysplasia were excluded from the study. Moreover, premature infants having additional systemic diseases or receiving additional treatments other than routine treatment were also excluded. Only the subjects with ROP at less than or equal to stage 3 were included since during the enrollment period, the numbers of patients with ROP at stages 4 and 5 were only 3 and 2, respectively, which were very low to include in the statistical analysis.

Subjects were classified into 2 groups: patients with ROP (n = 48) and patients without ROP (n = 23). ROP was determined by an examination 4–6 weeks after birth.[12] Pupillary dilatation and indirect ophthalmoscopy was used to determine ROP manifestation and severity. At the first ROP screening, a single aliquot of peripheral venous blood (1 mL) was collected.

Venous blood samples were separated, by centrifugation, into serum and plasma; serum was stored at -80°C until analysis. Serum levels of native and total thiol as well as the ratio of disulfide to native and total thiol were analyzed by an automated colorimetric method developed by Erel and Neselioglu.[9] In this new method, which is used in biochemical analysis to assess serum thiol-disulfide homeostasis, the disulfide bonds (-S-S-) are reduced to functional thiols (-SH) with NaBH4, followed by NaBH4 removal with formaldehyde. Ellman's reagent was used to analyze total sample thiol amounts. The dynamic disulfide content was calculated as a ratio of half of the difference between the total and native thiol. Thus, the native thiol, total thiol, and disulfide levels were measured and the percentages of disulfide-to-native, disulfide-to-total, and native-to-total thiol were calculated for all patients with and without ROP.[9]

STATISTICAL ANALYSIS

The data were analyzed using the IBM SPSS Statistics version 21.0 (IBM Corp., Chicago, IL, USA). The normality of each variable was controlled with the Kolmogorov-Smirnov test. The student t-test and the Mann-Whitney U test were used for parametric data and nonparametric data for group comparison, respectively. A one-way analysis of variance (ANOVA) test for parametric data and Kruskal-Wallis test for nonparametric data were used to compare the measures among subgroups according to stages of ROP. A p value less than 0.05 was considered statistically significant.

RESULTS

Forty eight ROP subjects (26 boys and 22 girls) and 23 control subjects (12 boys and 11 girls) were recruited for the study. The mean weights were 1,075.0 ± 150.82 g for subjects with ROP and 1,107.82 ± 126.34 g for subjects without ROP.

The mean gestational age was 28.85 ± 1.36 weeks for ROP patients and 29.43 ± 0.99 weeks for controls. There were no significant differences between the two groups according to sex, mean weights, and gestational age (p = 0.875, p = 0.051, and p = 0.062, respectively). Table 1 shows the demographic features of each group.

Comparison of thiol-disulfide measurements between the ROP patients and controls are demonstrated in Table 2 and Figure 1. When we analyzed the thiol/disulfide homeostasis parameters, no significant differences were observed between the ROP patients and controls in terms of serum native thiol (p = 0.913) and total thiol (p = 0.420); however, native-to-total thiol ratio was significantly lower in the ROP patients (p = 0.017). On the other hand, ROP patients had significantly higher level of disulfide as well as disulfide-to-native thiol and disulfide-to-total thiol ratios as compared with the controls (p < 0.001, p = 0.017, and p = 0.017, respectively). The differences in all thiol/disulfide homeostasis parameters (except for native and total thiol) between the ROP patients and controls were statistically significant.

There were no significant differences among the ROP patients at stage 1 (n = 18), stage 2 (n = 19), and stage 3 (n = 11) regarding the thiol/disulfide homeostasis parameters (p = 0.575 for native thiol, p = 0.808 for total thiol, p = 0.061 for disulfide, ANOVA; p = 0.129 for disulfide-to-native thiol, p = 0.129 for disulfide-to-total thiol, p = 0.129 for native thiol /total thiol, Kruskal-Wallis test).

DISCUSSION

Knowledge, diagnosis, and management of ROP have improved since the origin of high oxygen associated retrolental fibroplasia.

With improvements in neonatal care, several changes in our knowledge of ROP have occurred. New therapies to encourage physiologic retinal vascular development, vascular repair, and inhibition of vasoproliferation have been recommended.

Reducing excessive oxidative/nitrosative stress has also been studied. OS happens when the generation and quenching of reactive oxygen species are not at homeostasis. Considerable evidence supports the view that OS has an important etiological role in ROP pathogenesis.[13-17]

Thiol/disulfide balance is a localized and systemic OS gauge. It has a crucial role for several cellular functions, including antioxidant protection. The valuation of OS through thiol/disulfide homeostasis assay has become a topic of interest for multiple diseases. Various systemic pathologies can result from an imbalance of thiol-disulfide homeostasis, such as diabetes[18] and cardiovascular diseases.[19] Recently, many localized diseases have been associated with a deterioration in thiol-disulfide homeostasis, such as central serous chorioretinopathy,[20] age-related macular degeneration,[21] seasonal allergic rhinitis,[22] and acute tonsillopharyngitis.[23]

To the best of our knowledge, the relationship between thiol/disulfide homeostasis and ROP has not been

Table 1. *Demographic features of the study groups.*

Parameters	Patients with ROP (n = 48)	Patients without ROP (n = 23)	p value
Gestational age, weeks	28.85 ± 1.36	29.43 ± 0.99	0.062
Sex, girls / boys	22/26	11/12	0.875
Birth weight, g	1,075.0 ± 150.82	1,107.82 ± 126.34	0.051
ROP = Retinopathy of prematurity			

Table 2. *Plasma thiol-disulfide levels of groups.*

Parameters	Patients with ROP		Patients without ROP		p value
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
Native thiol, µmol/L	159.60 ± 44.68	160 (134.0–195.4)	160.82 ± 41.02	156.3 (132.6–191.8)	0.913
Total thiol, µmol/L	198.93 ± 44.65	199.1 (170.0–227.2)	189.76 ± 44.56	180.5 (159.0–225.9)	0.420
Disulfide, µmol/L	19.66 ± 7.44	18.7 (14.2–23.6)	14.46 ± 3.42	15.4 (11.9–17.0)	**<0.001**
Disulfide /Native thiol, %	6.93 ± 4.62	5.8 (3.8–8.1)	4.68 ± 1.42	4.3 (3.7–5.8)	**0.017**
Disulfide /Total thiol, %	5.11 ± 2.23	4.7 (3.3–6.1)	3.90 ± 0.97	3.7 (3.2–4.7)	**0.017**
Native thiol /Total thiol, %	79.53 ± 8.94	80.9 (75.3–86.5)	84.39 ± 3.88	85.1 (81.0–87.1)	**0.017**
ROP = Retinopathy of prematurity; IQR = Interquartile range; SD = Standard deviation					

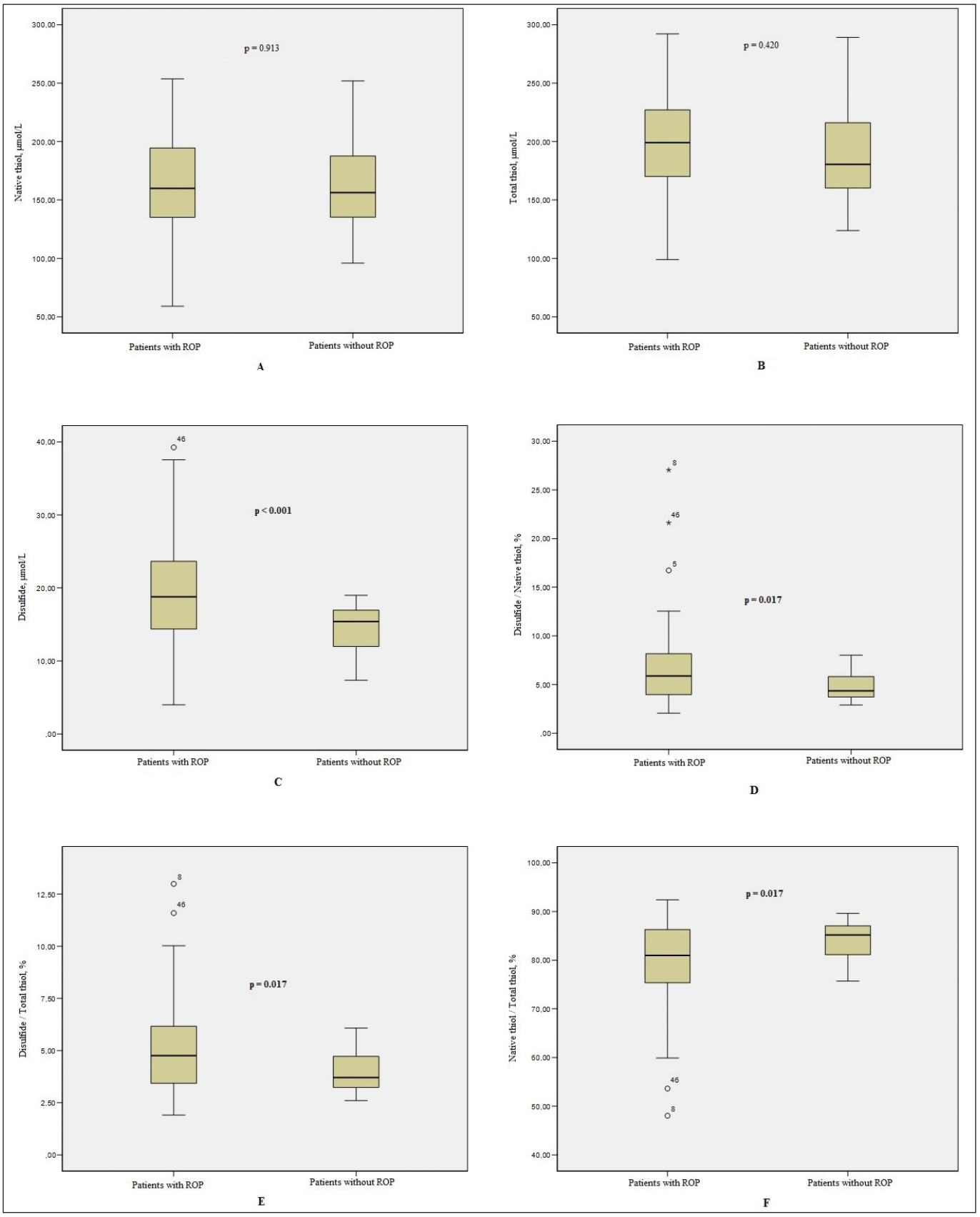

Figure 1. *Comparison of thiol-disulfide measurements between the retinopathy of prematurity patients and controls in terms of:* (**A**) *Native thiol;* (**B**) *Total thiol;* (**C**) *Disulfide;* (**D**) *Disulfide / Native thiol ratio;* (**E**) *Disulfide / Total thiol ratio; and* (**F**) *Native thiol / Total thiol ratio.*

previously studied. In the present study, our goal was to determine thiol/disulfide homeostasis in the ROP patients in comparison with the patients without ROP using the novel colorimetric method developed by Erel and Neselioglu.[9] We found that native-to-total thiol ratios were lower in the blood of ROP patients. There were no significant differences for native thiol and total thiol values in the ROP patients as compared to the controls. Moreover, our study found that disulfide, disulfide-to-native thiol ratios, and disulfide-to-total thiol ratios were higher in the ROP patients than those in the controls. We suggest that decreased antioxidant status in the ROP patients has a role in this thiol/disulfide imbalance. Although there is no study in the literature that we can directly compare our findings, the findings of Ünal et al.[24] are valuable for this thiol/disulfide imbalance. They evaluated the dynamic thiol/disulfide homeostasis in very low birth weighted preterms at baseline, 1st week, and 3rd week. They found that thiol levels increased in all measurements; disulfide and disulfide/thiol ratio increased at the 1st week but decreased at the 3rd week, and native-to-total thiol ratio decreased at the 1st week but increased at the 3rd week. Therefore, they attributed this shift towards disulfides in the thiol/disulfide homeostasis in the 1st week to the subjection of patients to OS. In premature infants and ROP, altered oxygen, light, and metabolism have been linked to higher OS. Pre-term labor and pre-term birth can decrease the oxidative reserve and present higher OS in comparison to full-term.[25] On the other hand, Akdoğan et al.[26] conducted a study on 28 infants with ROP at stage 2 and higher to determine the effects of a potent antioxidant on thiol/disulfide homeostasis. However, they found no differences in the total thiol, native thiol, and disulphide levels as well as native/total thiol ratio and disulphide/total ratio after two weeks of antioxidant administration.

Mounting evidence has shown that NADPH oxidase is important in experimental oxygen-induced retinopathy models. This method works to generate reactive oxygen species that restrict peripheral retina vascularization and mediation of later vasoproliferation into the vitreous.[27]

Hyperoxia-induced apoptosis happens via nitrosative stress from peroxynitrite.[28-30] The formation of peroxynitrite from nitric oxide occurs in the presence of increased superoxide radicals. Arginase-2 has been demonstrated for its role in peroxynitrite-mediated neuro-glial injury[31] and vaso-obliteration in oxygen-induced retinopathy.[32]

In previous report, Ateş et al. found higher mean plasma and urine 8-hydroxy 2-deoxyguanosine and malondialdehyde levels in patients with ROP compared to controls, suggesting that 8-hydroxy 2-deoxyguanosine in leukocyte DNA and urine may be a useful biomarker for the evaluation of oxidative damage in patients with ROP.[33]

The use of anti-oxidants, including superoxide dismutase in liposomes[34] or apocynin,[35] reduced avascular retina in phase 1 of the rat oxygen-induced retinopathy model but these substances did not reduce intravitreous neovascularization in phase 2 in the rat oxygen-induced retinopathy model.

Antioxidants may be effective for blocking reactive oxygen species, but may not have the ability to affect those that trigger pathologic intracellular signaling molecules. Studies have expanded our knowledge on the oxidative and nitrosative compounds in ROP and show potential as future treatment options. Oxidative/nitrosative balance is necessary and more studies on the matter are warranted.[16]

The main limitation of our study is its systemic evaluation of thiol/disulfide homeostasis. Our results do not indicate that the OS increased in vitreous. Ideally, thiol disulfide levels should be measured from vitreous. However, using this technique is not presently possible, since most thiol molecules are located in albumin. The next limitation of the study is the lack of comparison between these parameters and the patients in severe stages of (stage 4 and stage 5) ROP and controls. More longitudinal analyses are required for precise delineation of these implications in severe ROP patients.

In conclusion, our results showed an impaired systemic thiol/disulfide homeostasis in the ROP subjects compared to premature infants without ROP. This imbalance suggests the role of decreased systemic antioxidant status in ROP development.

REFERENCES / KAYNAKLAR

1. Terry TL. Extreme prematurity and fibroblastic overgrowth of persistent vascular sheath behind each crystalline lens: I. Preliminary report. Am J Ophthalmol 1942;25:203-4.
2. Gilbert C. Retinopathy of prematurity: A global perspective of the epidemics, population of babies at risk and implications for control. Early Hum Dev 2008;84:77-82.
3. Hartnett ME, Penn JS. Mechanisms and management of retinopathy of prematurity. N Engl J Med 2012;367:2515-26.
4. Stone WL, Farnsworth CC, Dratz EA. A reinvestigation of the fatty acid content of bovine, rat and frog retinal rod outer segments. Exp Eye Res 1979;28:387-97.
5. Stone WL, Katz ML, Lurie M, et al. Effects of dietary vitamin E and selenium on light damage to the rat retina. Photochem Photobiol1979;29:725-30.
6. McColm JR, Geisen P, Hartnett ME. VEGF isoforms and their expression after a single episode of hypoxia or repeated fluctuations between hyperoxia and hypoxia: Relevance to clinical ROP. Mol Vis 2004;10:512-20.
7. Wang H, Yang Z, Jiang Y, et al. Endothelial NADPH oxidase 4 mediates vascular endothelial growth factor receptor 2-induced intravitreal neovascularization in a rat model of retinopathy of prematurity. Mol Vis 2014;20:231-41.

8. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. Free Radic Biol Med 2009;47:1329-38.
9. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32.
10. Biswas S, Chida AS, Rahman I. Redox modifications of protein–thiols: emerging roles incell signaling. Biochem Pharmacol 2006;71:551-64.
11. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 2010;48:749-62.
12. American Academy of Pediatrics. Section on Ophthalmology. Screening examination of premature infants for retinopathy of prematurity. Pediatrics 2001;108:809-11.
13. Rivera JC, Sapieha P, Joyal JS, et al. Understanding retinopathy of prematurity: update on pathogenesis. Neonatology 2011;100:343-53.
14. Tan SM, Stefanovic N, Tan G, et al. Lack of the antioxidant glutathione peroxidase-1 (GPx1) exacerbates retinopathy of prematurity in mice. Invest Ophthalmol Vis Sci 2013;54:555-62.
15. Li SY, Fu ZJ, Lo AC. Hypoxia-induced oxidative stress in ischemic retinopathy. Oxid Med Cell Longev 2012;2012:426769.
16. Wang H,Zhang SX,Hartnett ME. Signaling pathways triggered by oxidative stress that mediate features of severe retinopathy of prematurity. JAMA Ophthalmol 2013;131:80-5.
17. Wilkinson-Berka JL, Rana I, Armani R, et al. Reactive oxygen species, Nox and angiotensin II in angiogenesis: implications for retinopathy. Clin Sci (Lond) 2013;124:597-615.
18. Matteucci E, Giampietro O. Thiol signalling network with an eye to diabetes. Molecules 2010;15:8890-903.
19. Go YM, Jones DP. Cysteine/cystine redox signaling in cardiovascular disease. Free Radic Biol Med 2011;50:495-509.
20. Turkoglu EB, Dikci S, Çelik E, et al. Thiol/disulfide homeostasis in patients with central serous chorioretinopathy. Curr Eye Res 2016;41:1489-91.
21. Arıkan Yorgun M, Toklu Y, Altınkaynak H, et al. A novel tool for the assessment oxidative stress in age-related macular degeneration: thiol/disulfide homeostasis revisited. Curr Eye Res 2016;41:1584-9.
22. Ulusoy S, Ayan NN, Dinc ME, et al. A new oxidative stress marker for thiol-disulphide homeostasis in seasonal allergic rhinitis. Am J Rhinol Allergy 2016;30:53-7.
23. Kara SS, Erel O, Demirdag TB, et al. Alteration of thiol-disulphide homeostasis in acute tonsillopharyngitis. Redox Rep 2017;22:205-9.
24. Unal S, Ulubas Isik D, Bas AY, et al. Evaluation of dynamic thiol-disulfide homeostasis in very low-birth-weighted preterms.J Matern Fetal Neonatal Med 2019;32:1111-6.
25. Buhimschi IA, Buhimschi CS, Pupkin M, et al. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. Am J Obstet Gynecol 2003;189:181-8.
26. Akdogan M, UstundagY, Akdağ A, et al. The thiol-disulfide homeostasis and coenzyme Q10 in conjunction with vitamin E effect on retinopathy prematurity. The Open Ophthalmology Journal 2019;13:23-8.
27. Saito Y, Uppal A, Byfield G, et al. Activated NAD(P)H oxidase from supplemental oxygen induces neovascularization independent of VEGF in retinopathy of prematurity model. Invest Ophthalmol Vis Sci 2008;49:1591-8.
28. Brooks SE, Gu X, Samuel S, et al. Reduced severity of oxygen-induced retinopathy in eNOS-deficient mice. Invest Ophthalmol Vis Sci 2001;42:222-8.
29. Gu X, El-Remessy AB, Brooks SE, et al. Hyperoxia induces retinal vascular endothelial cell apoptosis through formation of peroxynitrite. Am J Physiol Cell Physiol 2003;285:C546-54.
30. Ishida S, Yamashiro K, Usui T, et al. Leukocytes mediate retinal vascular remodeling during development and vaso-obliteration in disease. Nat Med 2003;9:781-8.
31. Narayanan SP, Suwanpradid J, Saul A, et al. Arginase 2 deletion reduces neuro-glial injury and improves retinal function in a model of retinopathy of prematurity. PLoS One 2011;6:e22460.
32. Suwanpradid J, Rojas M, Behzadian MA, et al. Arginase 2 deficiency prevents oxidative stress and limits hyperoxia-induced retinal vascular degeneration. PLoS One 2014;9:e110604.
33. Ates O, Alp HH, Caner I, et al.OxidativeDNA damageinretinopathy of prematurity. Eur J Ophthalmol 2009;19:80-5.
34. Niesman MR, Johnson KA, Penn JS. Therapeutic effect of liposomal superoxide dismutase in an animal model of retinopathy of prematurity. Neurochem Res 1997;22:597-605.
35. Saito Y, Geisen P, Uppal A, et al. Inhibition of NAD(P)H oxidase reduces apoptosis and avascular retina in an animal model of retinopathy of prematurity. Mol Vis 2007;13:840-53.