Oxidative Stress Under Phototherapy

Fototerapinin Neden Olduğu Oksidatif Stres

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ABSTRACT Jaundice is common among newborn babies and phototherapy (PT) is frequently used to treat pathologic hyperbilirubinemia. Although it is considered to be safe, the effects of PT on antioxidant systems are not well documented. The aim of this study was to investigate possible changes in antioxidant systems of full term otherwise healthy neonates during PT. A total of 111 newborn babies were included in this study. Fifty-six term infants who needed PT due to indirect hyperbilirubinemia constituted the patient group and 55 healthy term infants formed the control group. Levels of malondialdehide (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), reduced glutathione (GSH), vitamin E, total antioxidant capacity (TAC) were measured before and after PT in patient group and during routine screening tests in control group. TAC significantly decreased after PT (1.42 ± 0.22 vs 1.29 ± 0.27 mmol/L) in patient group (p= 0.001). TAC was significantly higher in patients before PT compared to control group (1.42 ± 0.22 vs 1.02 ± 0.29 mmol/L) (p< 0.001). GPX was significantly lower and MDA was found significantly higher after PT in patient group (p= 0.009, p= 0.002). SOD, GSH and vitamin E remained unchanged after PT. In conclusion, PT thought to cause oxidative stress in term babies with jaundice. This effect of PT should be taken in to consideration during the management of infants with hyperbilirubinemia and unnecessary usage should be avoided.

Key Words: Infant, newborn; hyperbilirubinemia; oxidative stress; antioxidants; malondialdehyde

ÖZET Hiperbilirubinemi, yenidoğan bebeklerde sık görülen bir durumdur ve patolojik hiperbilirubineminin tedavisinde fototerapi (FT) yaygın olarak kullanılmaktadır. Yenidoğan bebeklerin FT'ye maruz kalmasının antioksidan sistemleri üzerine olan etkileri hakkında çelişkili sonuçlar bildirilmiştir. Bu çalışma sağlıklı, zamanında doğmuş yenidoğan bebeklerde, FT'nin antioksidan sistemler üzerindeki etkilerini değerlendirmek amacıyla yapılmıştır. Çalışmaya indirek hiperbilirübinemi nedeni ile FT uygulanan 56 yenidoğan bebek (hasta grubu) ve poliklinikte takip edilen, zamanında doğmuş 55 sağlıklı bebek (kontrol grubu) olmak üzere toplam 111 yenidoğan bebek alındı. Hasta grubundaki bebeklerden FT öncesi ve sonrasında, kontrol grubundaki bebeklerden ise rutin taramalar sırasında serum malon dialdehid (MDA), süperoksit dismutaz (SOD), glutatyon peroksidaz (GPX), redükte glutatyon (GSH), E vitamini ve total antioksidan kapasite (TAK) düzeyleri ölçüldü. Hasta grubunda FT öncesi serum TAK düzeyi (1.42 ± 0.22 mmol/L), FT sonrasında bulunan düzeyden (1.29 ± 0.27 mmol/L) ve kontrol grubundaki bebeklerdekinden (1.02 ± 0.29 mmol/L) anlamlı olarak daha yüksekti (sırasıyla p= 0.001 ve p< 0.001). Hasta grubunda, FT öncesi değerlerle karşılaştırıldığında, FT sonrasındaki serum GPX düzeyi anlamlı olarak daha düşük, serum MDA düzeyi anlamlı olarak daha yüksekti (p= 0.009, p= 0.002), ancak diğer parametreler FT öncesi ve sonrasında farklı değildi. Bu sonuçlar FT'nin oksidatif bir strese neden olabileceğini düşündürmektedir. FT uygulamalarında bu etkinin göz önünde bulundurulması ve gereksiz kullanımdan kaçınılması gerektiği kanısına varılmıştır.

Anahtar Kelimeler: Yenidoğan; hiperbilirubinemi; oksidatif stres; antioksidanlar; malondialdehid

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Bilirubin is considered as a scavenger of reactive oxygen species and protects the neonate from oxygen radical diseases.[1] Unconjugated bilirubin is able to scavenge single oxygen, to react with superoxide anions and peroxyl radicals and to serve as a reducing substrate for peroxidases.[2] Reactive oxygen species are produced during oxidative reactions which normally take place in human body. Production and elimination of these molecules are critically balanced by oxidative stimulus and antioxidant defense mechanisms.[3] Tissue oxygen concentration is known to be low during intrauterine life, but babies are subjected to higher oxygen concentration soon after birth which may cause oxidative damage by the increase of free radicals. Neonates, particularly the preterm infants, have limited antioxidant capacity against reactive oxygen species during perinatal period.[4] Any procedure causing oxidative stress may produce highly reactive oxygen species which can disrupt cell membrane integrity by lipid peroxidation. These reactions may lead tissue injury and more than 100 disease states like bronchopulmonary displasia, retinopathy of prematurity, intraventricular hemorrhage and necrotizing enterocolitis.[5-6]

Jaundice is a common clinical condition among neonates. Great majority of newborn babies, particularly preterm infants, experience some degree of hyperbilirubinemia. Although bilirubin is a powerful component of antioxidant capacity, it may cause neurological damage at high levels and should be treated promptly to decrease the risk of kernicterus. PT is widely used in the treatment of neonatal jaundice which is thought to have a negative effect on antioxidant capacity by reducing serum total bilirubin level. During PT bilirubin is converted into water soluble photoisomers by structural isomerization and photo-oxidation. A number of photoproducts are generated during this procedure, leading to oxidative stress. The degree of oxidative stress may be estimated by measuring the levels of antioxidant enzymes, antioxidant molecules and oxidant markers. The relationship between PT and antioxidant enzymes such as SOD, catalase (CAT) and GPX; and antioxidant molecules such as albumin, seruloplasmine, ferritine, ascorbic acid, vitamin E, beta carotene, ubiquinol 10, GSH, methionine, uric acid and TAC is not well documented. The effect of PT on serum MDA that is the end product of lipid peroxidation and known as an oxidant marker is still not well established. The aim of this study was to investigate the effects of PT on the balance between oxidative stimuli and antioxidant defense mechanisms by measuring serum levels of MDA, SOD, CAT, GPX, GSH, vitamin E and TAC.

MATERIAL AND METHODS

The study was conducted in the neonatal intensive care unit of Mersin University Medical Faculty after approval by the local ethics committee. A total of 111 neonates were included and informed consent was obtained from the parents. Fifty-six term infants who needed PT with postnatal age of less than 15 days and a total bilirubin level over 15 mg/dL constituted the patient group. Fifty-five healthy, birth weight, gestational age and postnatal age matched term infants with a total bilirubin level less than 10 mg/dL served as controls. The babies with signs of hemolysis, who were not exclusively breastfed, who had hepatic or renal dysfunction, abnormal physical examination findings other than jaundice and with a history of perinatal asphyxia, congenital malformations, sepsis or other systemic diseases and drug use other than vitamin K were not included in the study.

All infants in the patient group received standard and continuous PT (450 nm, 13 $\mu W/cm^2/nm$; Micro-Lite Phototherapy System, Draeger) according to the guidelines of American Academy of Pediatrics Venous blood samples were taken into standard tubes and tubes containing ethylene diamine tetra acetic acid (EDTA) before and soon after PT. The blood samples which were taken into standard tubes were centrifuged and serum samples were stored at -20°C to measure MDA, vitamin E and TAC. Plasma samples obtained from the tubes with EDTA were stored at 4°C to measure CAT, SOD, GPX and GSH.

MDA, GSH and vitamin E concentrations were measured by HPLC (Chromsystems Instruments & Chemicals GmbH, München, Germany). CAT and SOD enzyme activities were measured by Beutler method and RANSOD kit (RANSOD SOD kit, Cat no. BT29+QY.56, Randox Lab. Ltd. Antrium, UK), respectively.[7] GPX enzyme activity was measured with RANSEL kit (RANSEL GSH-Px kit, Cat no: RS 504, Randox Lab. Ltd. UK) which is based on Paglia and Valentine method.[8] TAC, was measured with TAS manual kit (TAS manuel kit, Cat. no. NX 2332, Randox Lab. Ltd., Antrim, U.K).

STATISTICAL ANALYSIS

For statistical analysis, normal distribution of the values was assessed by Shapiro Wilk's Test. All data that show normal distribution were expressed as mean ± SD (standard deviation) and the others were expressed as median (25^{th}-75^{th} percentiles). The comparisons of the before and after PT values for MDA, vitamin E, CAT, SOD, GPX and GSH variables were done by using Wilcoxon Signed Ranks Test. The comparison of the before and after PT values for TAC variable was done by using paired samples t test. Mann-Whitney U test was used to compare groups separately for MDA, vitamin E, CAT, SOD, GPX and GSH values. Comparison between groups was done by using Independent samples t test for TAC values. Two-tailed p values less than 0.05 were regarded as significant. For statistically analyses, statistical packages of SPSS for Windows version 11.0 were used (SPSS, Inc., Chicago, IL, USA).

TABLE 1: Demographic parameters of the patient and control groups.

	Patient Group (n= 56)	Control Group (n= 55)	p
Gestational Age (week)	38.1± 0.9	38.5 ± 1.1	0.555
Age (day)	4.6 ± 2.1	5.2 ± 2.1	0.097
Weight (gram)	3328 ± 530	3487 ± 640	0.156
Gender (Female/Male) (%)	39.3/60.7	50.9/49.1	0.222

RESULTS

The mean gestational age, postnatal age and birth weight were not different in the patient and control groups (Table 1). Gender distribution was similar in the groups. The onset of jaundice was 2.4 days and the mean duration of PT was 40.1 hours in the patient group.

The serum TAC and GSH levels were significantly higher in the patient group before PT when compared to controls (1.42 vs 1.02 mmol/L, p< 0.001; 650.9 vs 397.5, p= 0.023 respectively) (Table 2). All other parameters were similar in the patient group before PT and the control group.

The serum GPX and serum TAC levels were found to be significantly decreased after PT compared to the levels obtained before PT in the patient group (p= 0.010, p= 0.001 respectively) (Table 3). The serum levels of MDA was found significantly increased after PT (p= 0.002). The median serum levels of vitamin E and the median plasma levels of CAT, SOD, GPX and GSH were not different in the patient group before and after PT (Table 3).

TABLE 2: Serum MDA and antioxidant levels in the patient (before PT) and control groups.

	Control Group (n= 55)	Patient Group, Before PT (n= 56)	p
Malondialdehyde (µmol/L)	0.185 (0.12-0.27)	0.153 (0.109-0.202)	0.098
Vitamin E (µmol/L)	22.1 (19-30)	19.5 (17-26)	0.118
Superoxide dismutase (U/gHb)	444.3 (307-600)	435 (313-647)	0.813
Catalase (IU/gHb)	10614 (9195-13054)	10248 (8906-11565)	0.306
Glutathione peroxidase (IU/gHb)	7344 (5040-10080)	7800 (4752-10740)	0.683
Reduced glutathione (µmol/L)	397.2 (213-817)	650.9 (338-1056)	0.023
Total Antioxidant Capacity (mmol/L)	0.99 (0.80-1.25)	1.41 (1.28±1.59)	<0.001

PT: Phototherapy

TABLE 3: Serum MDA and antioxidant levels in the patient groups.

	Patient Group (n= 56)		
	Before Phototherapy	After Phototherapy	p
Malondialdehyde (µmol/L)	0.153 (0.109-0.202)	0.170 (0.136-0.249)	0.002
Vitamin E (µmol/L)	19.5 (17-26)	22.29 (18.3-28.0)	0.152
Superoxide dismutase (U/gHb)	435 (313-647)	420 (291-593)	0.243
Catalase (IU/gHb)	10248 (8906-11565)	10248 (8906-11565)	0.429
Glutathione peroxidase (IU/gHb)	7800 (4752-10740)	6780 (4362-10143)	0.009
Reduced glutathione (µmol/L)	650.9 (338-1056)	695.7 (347-1028)	0.521
Total Antioxidant Capacity (mmol/L)	1.41 (1.28±1.59)	1.24 (1.11-1.44)	0.001

Complete blood count, reticulocyte count, liver and thyroid function tests were in the normal limits, direct coombs test was negative and signs of hemolysis were absent in the peripheric blood smear in all babies (Table 4).

TABLE 4: Routine laboratory tests in patients and control groups.

	Patient Group (n= 56)	Control Group (n= 55)
Total bilirubin (mg/dL)	18.3 ± 3.7	5.9 ± 3.6
Direct bilirubin (mg/dL)	0.6 ± 0.1	0.5 ± 0.2
AST (U/L)	29.1 ± 12.7	39.8 ± 17.2
ALT (U/L)	23.3 ± 14.7	27.7 ± 13.7
Hemoglobine (g/dL)	16.8 ± 2	15.5 ± 1.9
TT_4 µg/dL	12.1 ± 2.7	12.3 ± 2.1
TSH uU/mL	9.1 ± 6.9	7.4 ± 6.9
Reticulocyte (%)	1.4 ± 0.9	

DISCUSSION

The effects of PT on antioxidant system are not well documented and the role of PT as prooxidant is still controversial. While PT was blamed to cause oxidative stress in newborn infants in some studies others failed to prove such an effect.[9-11] The present study demonstrated that PT reduces TAC and GPX levels in term infants with hyperbilirubinemia which may cause a significant deficit in antioxidant defence mechanisms.

Oxidative reactions normally take place in all biological systems and free oxygen radicals are produced. Inactivation of these products is balanced by antioxidant system to let these compounds play their role without any toxic side effect. SOD, CAT and GPX are antioxidant enzymes that play important roles in cellular defence against oxidative injury.[12,13] Plasma GPX level was found to be significantly reduced after PT in our patient group which may cause a significant impairment in the elimination of hydrogen peroxide. Hydrogen peroxide is a toxic product which is proven to be responsible for DNA changes and cell injury.[14] GPX, together with SOD and CAT play a critical role in the elimination of hydrogen peroxide.[12] SOD and CAT levels remained unchanged after PT in our patient group. Results of the study by Akisu et al partly support our findings.[11] They evaluated 20 term and 16 preterm infants and found no change in the levels of SOD, CAT and GPX before and after PT and concluded that PT does not cause any oxidative stress neither in preterm nor in term babies. But a significant reduction in plasma GPX level in our study strongly suggests that PT has some prooxidant effects and is not as innocent as it is believed.

Unconjugated bilirubin is able to efficiently scavenge singlet oxygen. It serves as a reducing substrate for peroxydases in the presence of hydrogen peroxide and it reacts with superoxide anions and peroxyl radicals. A strong correlation was reported between serum TAC and indirect bilirubin concentration which indicates a strong antioxidant impact of bilirubin.[2,15-16] Moreover Gopinathan et al demonstrated that bilirubin contributes for 77.4% of the total antioxidant activity of plasma in jaundeced term neonates.[17] This finding led the authors to propose that moderataly increased plasma

bilirubin levels may be protective from oxidative stress. Our findings support this proposal. TAC was found to be significantly higher in the patient group before PT compared to controls and it significantly decreased after PT, emphasising the important role of bilirubin as an antioxidant.

Vitamin E is a lipid soluble antioxidant that prevents free radical formation derived from polyunsaturated free fatty acids.[12] Several studies have investigated the possible relationship between oxidative stress from PT and vitamin E but no relation was documented.[11,18] Similarly we were not able to show any relationship between PT and serum vitamin E levels.

Malondialdehyde is a highly reactive metabolite of free radical induced lipid peroxidation.[19] A positive correlation was reported between MDA and serum bilirubin levels in the presence of hemolysis but such a correlation was not reported in the absence of hemolysis.[20,21] Similarly we couldn't find any correlation between serum MDA and bilirubin levels in the present study. Results of the studies investigating the effect of PT on serum MDA levels are conflicting. Some investigators indicated that serum MDA concentrations significantly decreased after phototherapy, while others did not report any difference on serum MDA levels after PT.[20-22] Serum MDA levels were found to be significantly increased after PT in the present study. Although there is no specific explanation for these wide variations, we can speculate on timing of blood sampling for MDA measurement and the type of PT device. We measured serum MDA levels approximately 40 hours after starting PT and found serum MDA levels higher comparing the studies measured serum MDA levels 48-72 hours after starting PT. Serum MDA levels might be increasing in the early periods of PT suggesting on oxidative stress during this period. Antioxidative defence mechanisms may play a role in lowering serum MDA levels during late periods after starting PT.

In conclusion, our study demonstrated that the levels of total antioxidant capacity and GPX significantly reduced, the levels of MDA significantly increased in term infants with hyperbilirubinemia approximately 40 hours after PT. Increased risk of oxidative stress may be associated with PT. Such an effect of PT should be taken into consideration in the treatment of jaundice and unnecessary usage should be avoided.

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