ORIGINAL RESEARCH

Comparison of total oxidant/antioxidant status in unconjugated hyperbilirubinemia of newborn before and after conventional and LED phototherapy: A prospective randomized controlled trial

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Abstract

Purpose: We evaluated and compared the oxidant and antioxidant status of hyperbilirubinemic infants before and after the two forms of phototherapy: conventional and LED phototherapy, in order to identify the optimal treatment method.

Method: Thirty newborns exposed to conventional (Group I) phototherapy and 30 infants exposed to LED phototherapy (Group II) were studied. The serum total antioxidant capacity (TAC) and the total oxidant status (TOS) were assessed by EREL's method.

Results: There were no statistically significant differences in TAC or TOS levels between Group I and Group II prior to phototherapy, and no statistically significant difference in TAC levels between the two groups after phototherapy; however, TOS levels were significantly lower in Group II compared to Group I after phototherapy. Oxidative stress index (OSI) increased after conventional phototherapy

($p < 0.05$)

Conclusion: The increase in TOS following conventional phototherapy was not observed following LED phototherapy. This difference should be considered when using phototherapy.

Most newborns become clinically jaundiced during the first week of life. Bilirubin is normally cleared from the body by hepatic conjugation with glucuronic acid and eliminated in bile in the form of bilirubin glucuronides, but since the neonatal liver can not adequately clear bilirubin, bilirubin accumulation is observed. High levels of bilirubin are toxic to the central nervous system, so rapid intervention is necessary.

The most widely used method for lowering bilirubin levels is phototherapy, which uses light energy to change the molecular confirmation of bilirubin.¹⁻³ The resultant products are less lipophilic than bilirubin, and can be excreted in bile or urine without conjugation. Bilirubin absorbs light most strongly in the blue region of the spectrum near 460 nm, and the beneficial effect of phototherapy is dependent on the intensity and wavelengths of the light used. Recently, high intensity gallium nitride light emitting diodes (LEDs) have been developed and have generated significantly higher light irradiance levels in comparison with conventional phototherapy units.⁴⁻⁶ Efficacy of phototherapy is dependent on the wavelength of light emitted during phototherapy, the exposed body surface area and the duration of exposure.⁷ The American Academy of Pediatrics defines intensive phototherapy as irradiation of at least 30 $\mu\text{W}/(\text{cm}^2/\text{nm})$ in the 430-490 nm band.^{8,9}

Oxidants play an important role in the pathogenesis of many diseases and it is known that neonates have limited antioxidant protective capacity against circulating free radicals.¹⁰ A fetus is in a low oxygenation state and exposure to oxidative damage soon after birth, due to an immature antioxidant defence system can be especially detrimental.^{11,12}

It has recently been demonstrated that phototherapy produces oxidative stress. This is of particular concern for newborns, as antioxidant activity in the serum of term neonates is lower than that of adults, and the neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominantly pro-oxidant potential. Conventional phototherapy has been shown to have a negative impact on oxidant/antioxidant defence system and leads to increased oxidative stress in hyperbilirubinemic full term infants;¹³ however, the effect of LED phototherapy on oxidant and antioxidant status have never been studied before. In this report we evaluated and compared the oxidant and antioxidant status of hyperbilirubinemic infants before and after the two forms of phototherapy

to identify the therapeutic technique that best addresses elevated levels of oxidative stress.

Methods

This was a prospective randomized controlled clinical trial conducted in a tertiary neonatal intensive care unit in Turkey, from May 2009 to March 2010. The study was approved by the local Research Ethics Committee. All parents signed an informed consent form for the participation of newborns in the study.

Patients

Sixty healthy, term and late-preterm (≥ 35 weeks) newborn infants who were born at Zekai Tahir Burak Maternity Teaching Hospital and who exhibited clinically significant indirect hyperbilirubinemia requiring phototherapy in the first week of life comprised the subjects of the study. All infants were appropriate for gestational age (estimated by the last menstrual period and confirmed by ultrasound scan), were breast fed and had no pathologic etiological factors for hyperbilirubinemia. Infants with normal blood counts and peripheral blood smears, normal reticulocyte count, no evidence of blood group iso-immunization, negative result of a direct Coombs test, and normal glucose-6-phosphate dehydrogenase activity were eligible for the study. Infants with severe congenital malformation, positive direct Coombs test, enclosed hemorrhage, maternal diabetes, maternal eclampsia-preeclampsia, birth asphyxia, sepsis, hemolytic type of hyperbilirubinemia due to blood group or Rh incompatibility and those in whom the total serum bilirubin (TSB) level rose by more than 5 mg/dl per day or was higher than 20 mg/dl within the first 24 hours after birth were excluded from the study. The type of the phototherapy used was randomly assigned by the neonatal staff. Thirty infants were exposed to conventional phototherapy (Group I) and 30 infants to LED phototherapy (Group II).

Phototherapy

Criteria for starting phototherapy were based on American Academy of Pediatrics Practice Parameters as 25-48 hour serum total bilirubin levels: 15 mg/dl; 49-72 h: 17 mg/dl; >72 h: >17 mg/dl (8, 9). All infants were unclothed except for their eyes and genital region. All infants were exposed to continuous phototherapy, except while feeding and cleaning. The infants' weights and temperatures were monitored. All infants' gestational age, sex, birth weight, age at phototherapy, TSB level at initiation and termination of phototherapy, type of phototherapy, duration of phototherapy, blood type of the mothers and infants and direct Coombs test results were recorded.

For conventional phototherapy, the AMS Phototherapy System (intensity 12-16 $\mu\text{W}/\text{cm}^2/\text{nm}$, spectrum 430-470 nm, consisting of six fluorescent lamps) was used. For LED phototherapy, the Neoblu[®] LED phototherapy system (Natus Medical inc., San Carlos, CA, USA, intensity: 30 $\mu\text{W}/\text{cm}^2/\text{nm}$, spectrum 450-470 nm) was used. The system was placed over the infants, at a distance of 30 cm. The irradiance of the lamps was measured weekly and replaced if necessary. Phototherapy was stopped when two consecutive serum total bilirubin levels, measured 6 hours apart were below 2 mg/dl from the lowest limit for phototherapy.

Infants were carefully monitored for possible side effects of phototherapy, including dehydration, hypothermia, hyperthermia, skin rash and diarrhea.

Blood samples

Venous blood sampling (2 cc) was performed from a peripheral vein prior to phototherapy to determine total bilirubin, direct bilirubin, total antioxidant and oxidant capacity. Serum bilirubin levels were measured immediately but samples for total oxidant and antioxidant capacity were centrifuged at 1500 x g for 10 minutes within 30 minutes of collection, stored at -80°C, and analysed within 3 months. Serum total bilirubin levels were measured every 12 hours using a

direct spectrophotometry method. The bilirubinometer was calibrated daily and calibrated every 3 months against low and high bilirubin standards.

Second samples (2 cc blood) for the total antioxidant and oxidant concentrations were taken from a peripheral vein following phototherapy. Samples were centrifuged at 1500 x g for 10 minutes within 30 minutes of collection, stored at -80 °C, and analysed within 3 months.

Total antioxidant capacity (TAC) levels were measured by Erel's TAC method, which is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ATBS) radical cation by antioxidants. The results were expressed in mmol Trolox equiv/L. Serum thiol (total -SH group) content was measured by using dithionitrobenzoic acid (DTNB). Total oxidant status (TOS) serum concentrations were measured using Erel's TOS method, which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange. The results were expressed in $\mu\text{mol H}_2\text{O}_2/\text{L}$. Erel's TAC and TOS methods are colorimetric and automated and the precision of this assay is excellent - less than 3%. The TOS to TAC ratio was used as the oxidative stress index (OSI). The OSI value was calculated as follows: $\text{OSI} = [(\text{TOS}, \mu\text{mol /L})/(\text{TAS}, \mu\text{mol/L})/100]$ (14, 15, 16).

Statistical analysis

SPSS 16 for Windows[®] was used for statistics in the study. Statistical analyses were carried out using Student's t-test and chi-square test and the data were expressed as mean $\pm$ SD. A p-value of <0.05 was considered statistically significant.

Results

Sixty healthy, term and near term (>35 weeks) newborn infants were involved in the study. Each group consisted of 30 patients. There were no significant dif-

TABLE 1. Clinical and laboratory data of study groups

	Group I (n=30)	Group II (n=30)	P value
Gestational age (week)(mean±SD)	37.8 ±1.07	37.9±1.04	0.065
Birth weight (gr)(mean±SD)	3044±375	3044±364	0.639
Weight, at the start of phototherapy (gr)(mean±SD)	2999±379	3005±376	0.660
Age at the start of phototherapy, (h)	72±26	70±30	0.830
Duration of phototherapy, (h)	36±12	32±9	0.07
Sex (F/M)	19/11	15/15	0.297
TSB at the start of phototherapy (mg/dl) (mean±SD)	18±2.3	18.1±2.7	0.678
TSB level at the termination of phototherapy (mg/dl) (mean±SD)	11±1.4	9.9±1.7	0.082
Hematocrit, at the start of phototherapy (%)	51±6.2	52±4	0.064
Hematocrit, at the termination of phototherapy (%)	45±5	47±7	0.073

TSB: Total serum bilirubin, F: Female, M: Male

ferences in gestational week, birth weight, weight at the start of phototherapy, sex, TSB value before and after phototherapy, hematocrit levels at the start and termination of phototherapy and duration of phototherapy between the groups ($p>0.05$). All of the patients' direct Coombs test were negative, no hemolysis was observed on peripheral blood smears, and decreased hemotocrit was not observed in any patient. Clinical data and laboratory data are listed in Table 1.

No side effects of phototherapy were observed during the treatment. The infants tolerated the blue light well and no behavioral difference was observed between the infants exposed to fluorescent or LED light. Feeding was well tolerated and no vomiting or irritability was observed in either group. Phototherapy was effective in decreasing bilirubin levels in both groups, and no difference in TSB levels was observed at the end of the therapy. Serum antioxidant/oxidant parameters before and after conventional and LED phototherapy are shown in Table 2 and Table 3.

Discussion

All biological systems involve oxidative reactions, and toxic metabolites are often produced. The balance between the oxidative stimulus and the antioxidant defence mechanisms eliminate the toxic effects of the metabolites. As antioxidant mechanisms are immature in the neonatal period, this balance is unstable and circulating free radicals may cause intravascular hemolysis and lead to unconjugated hyperbilirubinemia.^{10,17} Phototherapy, although decreasing hyperbilirubinemia, may lead to oxidative injury to the red cell membrane and, as a result, increase the levels of lipid peroxidation products.¹⁸ Aycicek *et al.* showed that conventional phototherapy increases serum lipid hydroperoxides and has a negative impact on oxidant/antioxidant defence system in hyperbilirubinemic full term infants. They reported that TAC levels were not significantly altered by phototherapy but TOS and OSI levels were significantly increased in unconjugated hyperbilirubinemia.¹³ Atici *et al.* studied malondialdehyde (MDA), superoxide dismutase (SOD), glu-

TABLE 2. Comparison of oxidant and antioxidant serum parameters before and after conventional and LED phototherapy. Data are given as mean ±SD.

	TAC mmol Trolox equiv/L			TOS μmol H ₂ O ₂ /L		
	Group I	Group II	P	Group I	Group II	P
Before phototherapy	1.946±0.224	2.059±0.290	0.122	22.78±17.8	26.0±16.7	0.09
After phototherapy	1.900±0.205	1.963±0.084	0.06	51.69±25.7	26.55±14.3	<0.001

TAC: Total antioxidant capacity; TOS: Total oxidant stress

TABLE 3. Comparison of oxidant and antioxidant serum parameters before and after conventional and LED phototherapy. Data are given as mean $\pm$ SD.

	TAC			TOS			OSI		
	Before phototherapy (mmol Trolox equiv/ L)	After phototherapy	P	Before phototherapy (μ mol H ₂ O ₂ /L)	After phototherapy	P	Before phototherapy	After phototherapy	P
Group I	1.946 $\pm$ 0.224	1.900 $\pm$ 0.205	0.06	22.78 $\pm$ 17.8	51.69 $\pm$ 25.7	0.01	0.11	0.27	0.011
Group II	2.059 $\pm$ 0.290	1.963 $\pm$ 0.084	0.078	26.0 $\pm$ 16.7	26.55 $\pm$ 14.3	0.08	0.12	0.13	0.09

TAC: Total antioxidant capacity; TOS: Total oxidant stress; OSI: Total oxidant status

tathione peroxidase (GPX), reduced glutathione (GSH), vitamin E and total antioxidant capacity (TAC) levels before and after phototherapy. TAC was significantly higher in the patient group before phototherapy in comparison with the control group, and TAC significantly decreased after phototherapy in the patient group ($p=0.001$).¹⁸ These studies evaluated the conventional type of phototherapy but none of them compared conventional and LED phototherapy. In this study, we aimed to compare the total oxidant status and total antioxidant stress before and after conventional and LED phototherapy. We found that before the beginning of phototherapy, there were no statistically significant differences in TAC levels between Group I and Group II (1.946 $\pm$ 0.224 vs. 2.059 $\pm$ 0.290 mmol Trolox equiv/L), and there were no statistically significant differences in TOS levels between Group I and Group II (22.78 $\pm$ 17.8 vs. 26.0 $\pm$ 16.7 μ mol H₂O₂/L). After the phototherapy, TOS levels increases significantly in Group I (from 22.78 $\pm$ 17.8 to 51.69 $\pm$ 25.7 μ mol H₂O₂/L, $p=0.01$) but there was no statistically significant difference in TOS levels in Group II before and after phototherapy (from 26.0 $\pm$ 16.7 vs. 26.55 $\pm$ 14.3 μ mol H₂O₂/L $p=0.06$) (Table 2 and 3). In the LED-treated group the total antioxidant capacity did not decrease significantly, relative to the conventional phototherapy group. OSI index also increased after conventional phototherapy ($p<0.05$), indicating that oxidant stress increases after conventional phototherapy. The oxidant and antioxidant status after conventional phototherapy has been reported previously in the literature but this is the first study that evaluates the oxidant and antioxidant status after LED photo-

therapy and that compares it with conventional type of phototherapy.

In the study of Aycicek *et al.*, which evaluated the phototherapy and oxidative stress in full term infants, TOS before phototherapy was 11.34 $\pm$ 5.9 μ mol H₂O₂/L and reach a maximum of 16.34 $\pm$ 7.4 μ mol H₂O₂/L after phototherapy.¹³ In our study, TOS levels before phototherapy were 22.78 $\pm$ 17.8 and 26.0 $\pm$ 16.7 μ mol H₂O₂/L in group I and II, respectively; higher than the values reported in by Aycicek. There are a number of causes that might explain this difference. In Aycicek's study, only full term infants were enrolled in the study, but we evaluated both full and late preterm infants (>35 gestational weeks) in our study. In the literature there are a number of studies that show that oxidant stress is higher in preterms than term infants^{19,20} but there are no studies involving late preterms. The second cause might be the difference in placement of the phototherapy lamps: we put the phototherapy devices 30 cm above the infants, whereas Aycicek's group put them 40 cm above the infants. Since the light intensity is inversely related to the distance from the source and the relationship between intensity and distance is almost linear,²¹ this difference may account for the high TOS levels obtained in our study.

Although there are some studies in the literature that report different results about the duration of phototherapy, there was no significant difference between groups in our study. Karagol *et al.* reported in their study comparing conventional and LED phototherapy that duration of phototherapy in the patients who received LED phototherapy seemed shorter but the difference between the groups was not statistically significant.²² Additionally, two clinical trials of LEDs

did not report a higher efficacy when applied using relatively low irradiance levels^{14,18} Seidmann *et al.* compared the efficacy of LED phototherapy with an irradiance of >100 microwatts/cm²/nm to conventional phototherapy with 5-8 microwatts/cm²/nm and found that the mean TSB concentrations at initiation and termination of phototherapy treatment, and the duration of phototherapy, did not differ between newborns receiving LED and conventional phototherapy.^{23,24} Uras *et al.* compared LED and double standard conventional phototherapy for nonhemolytic anemia and found a marked decrease in bilirubin concentrations in the LED group, but that the duration of phototherapy was similar between the groups.²⁵ In this study, we did not aim compare the effect of different phototherapy types on duration of treatment, but the duration of treatment was similar between the two groups. As the demographic features of the patients, duration of the treatment, and distance of the lamps from the patient were similar for group I and II, we could not explain the difference of oxidant status by those variables. In conventional phototherapy, fluorescent lamps were used and the properties of the light might be the reason for high oxidant stress in conventional phototherapy group. LED lights have relatively low heat output, cause less change in the infants' thermal environment and less likely cause water loss, all of which are stress factors, in comparison with conventional phototherapy devices.

Previous studies examined the effect of phototherapy on serum total oxidant status, total antioxidant capacity, individual antioxidant components like vitamin C, uric acid, albumin, thiol components, bilirubin and individual oxidant components including malondialdehyde and lipid hydroperoxidase levels.^{13,20} Most of the published studies have investigated the oxidative effects of phototherapy and antioxidant enzyme activities, but not on the serum nonenzymatic total antioxidant capacity. Aycicek's study was the first that showed the association between these serum oxidant and antioxidant parameters in the phototherapy group.¹³ As the sum of the individual components

of oxidant and antioxidant capacity reflects the total oxidant and antioxidant status, in this study we evaluate both TAC and TOS levels rather than individual components.

Values for different oxidative stress markers in preterm and term newborns without unconjugated hyperbilirubinemia, have been reported in the literature. It is known that these levels vary according to infant weight (AGA, LGA and IUGR) but there are no reports or reference values for the late-preterm population without jaundice.^{26,27} In previous similar studies published by other authors, TAC and different oxidant and antioxidant components levels in a control group of full term newborns without hyperbilirubinemia, therefore not exposed to phototherapy, were studied,^{18,28} but we do not know the impact of preterm and late preterm gestational age on oxidant and antioxidant status. Additional studies should be planned in the future to clarify this.

One of the limitations of the study was that we did not calculate the ideal sample size before the beginning of the study: when we calculated the Roc curve retrospectively, the confidence interval was 95%. This ensures us that the sample size was large enough for this preliminary study. Studies with a larger sample size should be done in the future.

In conclusion, the increase in total oxidant status after conventional phototherapy was not observed after LED phototherapy. Therefore, we recommend very cautious use of conventional phototherapy in all patients of neonatal jaundice.

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