Pediatrics and Neonatology (2015) **56**, 95—100

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ORIGINAL ARTICLE

Investigation on Malondialdehyde, S100B, and Advanced Oxidation Protein Product Levels in Significant Hyperbilirubinemia and the Effect of Intensive Phototherapy on these Parameters

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Received Dec 5, 2013; received in revised form May 25, 2014; accepted Jun 19, 2014
Available online 26 September 2014

Key Words
advanced oxidation protein product;
hyperbilirubinemia;
intensive phototherapy;
malondialdehyde;
S100B protein

Background: The parameters of oxidative stress [advanced oxidation protein products (AOPPs), malondialdehyde (MDA), and S100B] and the effect of intensive phototherapy (PT) on these parameters have not been studied extensively in newborns with significant hyperbilirubinemia (SH). We aimed to measure the levels of MDA, S100B, and AOPPs in newborns with SH, and to compare newborns with healthy control newborns without hyperbilirubinemia on the basis of these parameters of oxidative stress. In addition, we investigated the effect of intensive PT on these parameters during the treatment of SH and report our findings for the first time in the literature.

Methods: The study was performed in newborns ($n = 62$) who underwent intensive PT because of SH. Newborns without jaundice constituted the control group ($n = 30$). Both groups were compared with respect to demographic characteristics and biochemical (laboratory) parameters including MDA, AOPPs, and S100B. MDA, AOPPs, and S100B were also compared before and after intensive PT in the PT group. In the study group, a correlation analysis of demographic characteristics; MDA, AOPP, and S100B values; and changes occurring in MDA, AOPPs, and S100B values due to the effect of intensive PT was performed.

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http://dx.doi.org/10.1016/j.pedneo.2014.06.006

Results: Serum total bilirubin, S100B, and MDA levels in the PT group before performing PT were significantly higher than those in the control group. In newborns receiving PT serum total bilirubin, MDA and AOPP levels decreased significantly after intensive PT. In correlation analysis, a statistically significant negative correlation was found only between the amount of bilirubin decrease with PT and AOPP levels after PT in the study group.
Conclusion: Whether the significant decrease in MDA levels, which was higher prior to PT, is due to the decrease in serum bilirubin levels or due to the effect of intensive PT itself remains to be determined in further studies. The decrease in AOPP levels after PT implies that intensive PT has protective effects on oxidative stress.

1. Introduction

In neonatology, free radicals and their products, lipid and protein peroxides, have been thought to be responsible for the pathogenesis of many conditions such as retinopathy of prematurity, bronchopulmonary dysplasia, intracranial hemorrhage, periventricular leucomalacia, sepsis, necrotizing enterocolitis, and hypoxic ischemic encephalopathy.[1] Bilirubin, at physiologic concentrations, protects neonatal red blood cells against oxidative stress. However, increased bilirubin concentrations are associated with significant cytotoxicity,[2] and significant hyperbilirubinemia (SH) may cause an increase in free radicals and their products.[2–4] On the other hand, phototherapy (PT) itself, which is the most commonly used treatment modality in SH, may also result in the release of reactive nitrogen and oxygen species, and photolysis products are cytotoxic and associated with the production of free oxygen radicals.[5] Ostrea et al[6] reported that the exposure of red cell suspension to PT light in the presence of a sensitizer (bilirubin) resulted in oxidative injury to the red cell membrane. Recently, *in vitro* fluorescent light has been shown to cause a decrease in red blood cell ATPase activity and an increase in lipid peroxidation, and it has been hypothesized that specific areas of the membrane, mainly the lipid/protein interface, are differently affected.[7] Various studies investigated the relationship between PT and/or SH and malondialdehyde (MDA) levels with conflicting results.[3,4,8–15]

Advanced oxidation protein products (AOPPs) are formed during oxidative stress,[1] and they are defined as novel parameters of oxidative stress.[16] There is a limited number of studies about AOPPs during the neonatal period, most of which have been conducted to investigate various free radical-mediated diseases.[1,17,18] However, to our knowledge, no studies have investigated the relationship between AOPPs and neonatal hyperbilirubinemia and/or PT.

S100B protein levels in cerebrospinal fluid, plasma, or serum have been used increasingly as biomarkers to evaluate the presence and severity of intraventricular hemorrhage, hypoxic ischemic encephalopathy, and brain damage.[19–21] Levels of S100B protein in neonatal SH were investigated in only one study,[22] and to our knowledge, the effect of PT has not yet been determined.

Thus, we aimed to measure the levels of MDA, S100B, and AOPPs in newborns with SH, and to compare these newborns with healthy control newborns without hyperbilirubinemia on the basis of these parameters of oxidative stress. In addition, we investigated the effect of intensive PT on these parameters during the treatment of SH for the first time in the literature.

2. Patients and methods

This study was performed on term and near-term ($\geq$35 weeks) newborns who underwent intensive PT because of SH between February 2011 and April 2013. A diagnosis of SH was made if the serum total bilirubin levels of the newborns were above the levels on age (hour)-specific bilirubin curves determined on the basis of risk status and gestational age of the newborns.[23,24] All infants were exposed (completely unclothed, with their eyes and genitals covered) to continuous PT that was interrupted only for feeding, cleaning, and blood sampling. The infants' weight and temperature were closely monitored during PT.

Sex, type of delivery, weight, length, and head circumference at birth, gestational age, age on admission, and maternal age of the newborns were recorded. Infants with any congenital malformation, prematurity ($<$35 weeks' gestation) or postmaturity, maternal diabetes, birth asphyxia, sepsis, or hemolytic-type hyperbilirubinemia due to blood group (Rh or ABO) incompatibility were excluded from the study. The study group comprised 62 newborns.

Intensive PT was chosen as the PT of choice in the treatment of SH, according to the guidelines of the American Academy of Pediatrics.[23] For PT, an intensive PT unit consisting of high-intensity gallium nitride light-emitting diodes (light spectrum = 450–470 nm and irradiance $\geq$ 35 $\mu W/cm^2/nm$; Bilicrystal IV. 2; Medestime S.A. Charleroi, Belgium) was used. The system was placed over the infants, at a distance of 40 cm. The irradiance was maintained above 35 $\mu W/cm^2/nm$ during PT and measured weekly. PT was stopped if serum bilirubin levels fell at least 5 mg/dL below the limits defined necessary for starting PT.

A control group (n = 30) was constituted by the newborns who had no jaundice, and were about to be discharged or came to the first control visit after hospital discharge. Blood samples from these cases were obtained during venipuncture performed for metabolic screening or various other reasons (such as control of thyroid function

tests and evaluation of hyperbilirubinemia). The study was approved by the ethics committee of the hospital, and written informed parental consent was obtained.

Blood samples of all cases were used to study the following parameters prior to PT (only once for the control cases irrespective of PT): blood group, Rh type, direct Coombs' test results, complete blood count, findings of liver function tests, serum total bilirubin, direct bilirubin, MDA, AOPPs, and S100B. Serum total bilirubin, MDA, AOPPs, and S100B were studied 24 hours after PT in the study (PT) group. Blood samples obtained for MDA, AOPPs, and S100B were stored at $-70^{\circ}C$ after centrifugation until analysis of all samples.

MDA levels in plasma were measured by the spectrophotometric method,[25] and the results were expressed as micromoles per liter (μmol MDA/L).

Determination of AOPPs was based on a spectrophotometric assay, according to Witko-Sarsat et al.[16] AOPP levels were expressed in terms of micromoles of chloramine-T equivalents per liter of plasma (μmol/L).

S100B protein concentrations were analyzed using a commercially available enzyme-linked immunosorbent assay kit (BioVendor Laboratory Medicine, A.S., Modrice, Czech Republic) according to the manufacturer's protocol. Results were expressed as pg/mL. Control and PT groups were compared with respect to demographic characteristics and biochemical (laboratory) parameters including MDA, AOPPs, and S100B. MDA, AOPPs, and S100B were also compared before and after PT in the PT group. In the study group, a correlation analysis of demographic characteristics; MDA, AOPP, and S100B values; and changes occurring in MDA, AOPPs, and S100B values due to the effect of PT was performed.

For statistical analysis, PASW Statistics 18.0 was used. One-sample Kolmogorov–Smirnov test was used to determine the distribution between groups. Independent sample *t* test was used to compare the PT and control groups. Paired sample *t* test was used to compare between the parameters before and after PT in the PT group. Pearson correlation analysis was performed to determine the correlation between demographic characteristics and biochemical (before and after PT) parameters. A value of $p \leq 0.05$ was accepted as statistically significant.

3. Results

Demographic characteristics and their statistical comparison in the study and control groups are given in Table 1. Sixty out of 62 newborns and 28 out of 30 newborns were being exclusively breast-fed in the study and control groups, respectively. There were no statistically significant differences between the study groups regarding the demographic characteristics. No newborns had glucose-6-phosphate dehydrogenase deficiency or required exchange transfusion as a further treatment modality in the study group.

Serum total bilirubin (8.1 ± 1.71 mg/dL vs. 20.58 ± 2.96 mg/dL; $p \leq 0.001$), S100B (87.3 ± 32.63 pg/mL vs. 124.97 ± 123.05 pg/mL; $p = 0.032$), and MDA levels (5.55 ± 0.6 nmol/mL vs. 7.72 ± 0.75 nmol/mL; $p \leq 0.001$) prior to PT were statistically significantly higher in the PT

Table 1 Comparison of the demographic characteristics of the study groups.

	Control group ($n = 30$)	Phototherapy group ($n = 62$)	*p*
Sex (male/female)	16/14	45/17	0.056
Type of delivery (vaginal/cesarean)	18/12	37/25	0.580
Birth weight (g)	3246 ± 287	3233 ± 396	0.873
Length (cm)	49.99 ± 0.7	49.79 ± 1.96	0.480
Head circumference (cm)	34.81 ± 0.57	34.7 ± 1.29	0.658
Gestational age (wk)	38.93 ± 0.98	38.58 ± 1.09	0.106
Age on admission (d)	5.23 ± 2.06	5.27 ± 2.15	0.933
Maternal age (y)	27.7 ± 5.16	29.19 ± 6.74	0.245

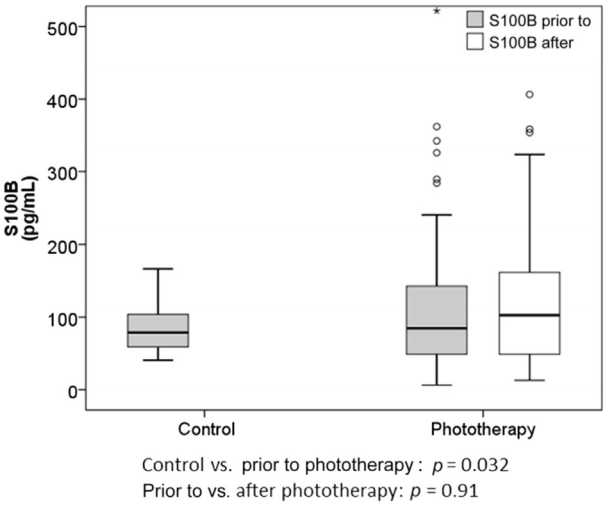

Figure 1 Comparison of the S100B levels in the control and phototherapy (before and after phototherapy) groups.

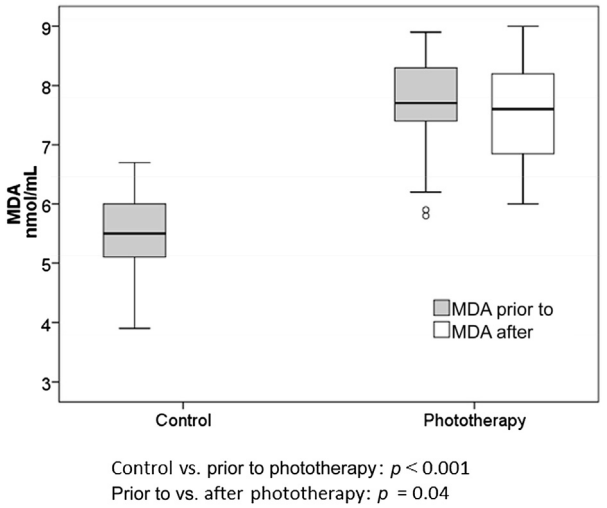

Figure 2 Comparison of the MDA levels in the control and phototherapy (before and after phototherapy) groups. MDA = malondialdehyde.

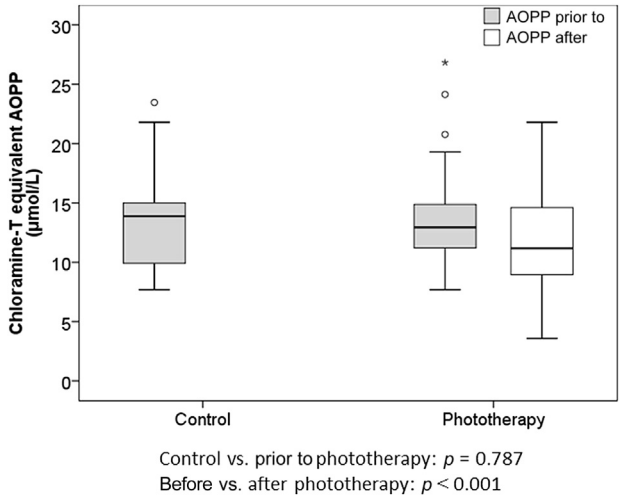

Figure 3 Comparison of the AOPP levels in the control and phototherapy (before and after phototherapy) groups. AOPP = advanced oxidation protein product.

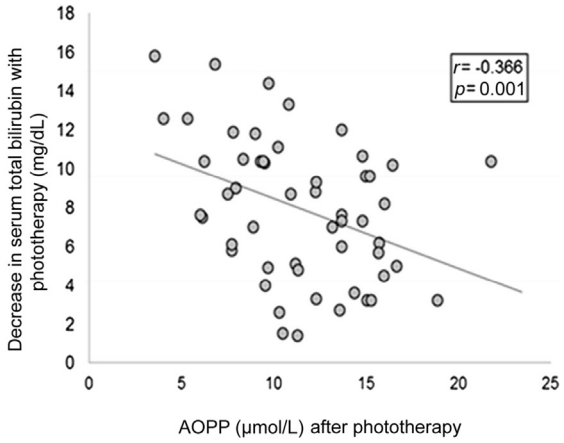

Figure 4 Correlation between the amount of bilirubin decrease with phototherapy and AOPP levels after phototherapy. AOPP = advanced oxidation protein product.

group than in the control group (Figures 1 and 2), whereas AOPP levels (13.42 ± 4.04 μmol/L vs. 13.19 ± 3.77 μmol/L; $p = 0.787$) did not differ significantly between the two groups (Figure 3).

In newborns receiving PT serum total bilirubin, MDA and AOPP levels decreased significantly after PT (Table 2, Figures 2 and 3), whereas S100B levels did not differ significantly due to the effect of PT in the PT group (Table 2 and Figure 1).

In correlation analysis of all the demographic and biochemical parameters, a statistically significant negative correlation was observed only between the amount of bilirubin decrease with PT and AOPP levels after PT in the study group, indicating that bilirubin decrease with PT was more prominent in cases with a more significant AOPP decrease [$r = -0.366$, $t = 0.001$ (Figure 4)].

4. Discussion

In this study, S100B levels were measured to investigate whether SH itself or intensive PT might cause a brain injury, as increased S100B levels correlate with a greater degree of brain damage. In the only other study with this aim, Okumus et al[22] evaluated 92 newborns with hyperbilirubinemia, and observed that serum Tau and S100B proteins began to increase when total serum bilirubin levels exceeded 19.1 mg/dL, and minor neurologic dysfunction, electroencephalogram abnormalities, and auditory neuropathy started developing at total serum bilirubin levels of 22 mg/dL, 24 mg/dL, and 25 mg/dL, respectively. They concluded that serum levels of Tau and S100B proteins were strongly correlated with early-phase bilirubin encephalopathy. In the present study, S100B levels were significantly higher in newborns with SH when compared to those without hyperbilirubinemia, confirming the finding of the study of Okumus et al.[22] However, S100B levels did not demonstrate a significant decrease after intensive PT, and the normalization period of S100B levels in newborns with SH should be followed with further longitudinal studies.

Increasing appreciation of the causative role of oxidative injury and lipid peroxidation in the development of many severe diseases of the newborn has lent tremendous importance to lipid peroxidation and its possible causes.[5] MDA is one of the most frequently used indicators of lipid peroxidation. Plasma MDA concentrations in neonates with SH were significantly higher than those in healthy infants.[3,4,8,10,11] Davutoglu et al[3] and Yigit et al[4] also reported a significant correlation between MDA and bilirubin levels. In contrast to these reports, only Kumar et al[9] found that jaundiced newborns had significantly lower MDA levels. In our study, MDA levels were significantly higher in newborns with SH requiring intensive PT in comparison to control cases without hyperbilirubinemia, confirming the finding reported by most of the related studies.[3,4,8,10,11]

There is a limited number of studies with controversial results comparing MDA levels before and after PT. Ozture et al[10] and Aycicek and Erel[13] reported that MDA concentrations decreased significantly with PT. Akisu et al[12] reported that MDA levels were not significantly different before and after PT in 20 full-term infants. However, Erdem

Table 2 Comparison of the biochemical (laboratory) characteristics before and after phototherapy in the study group ($n = 62$).

	Before phototherapy	After phototherapy	p
Serum total bilirubin level (mg/dL)	20.58 ± 2.96	12.49 ± 2.67	<0.001
S100B level (pg/mL)	124.97 ± 123.05	127.28 ± 94.92	0.91
Malondialdehyde level (nmol/mL)	7.72 ± 0.75	7.52 ± 0.79	0.04
Advanced oxidation protein product level (μmol/L)	13.19 ± 3.77	11.51 ± 3.77	<0.001

et al[14] and Dahiya et al[15] showed that MDA levels were higher after PT than before PT. In our study, MDA levels decreased significantly after intensive PT, in accordance with the results of the first two of these studies.

An imbalance between oxidant and antioxidant defense systems results in excessive reactive oxygen species generation and oxidative stress, which is associated with oxidative modification of proteins, lipids, and DNA, and leads to cell transformation or cell death by apoptotic or necrotic mechanisms.[26] AOPP is a novel marker of oxidant-mediated protein damage.[17] Recently, increased levels of AOPPs have been found in premature patients with free radical-related diseases,[1] necrotizing enterocolitis (NEC),[20] and hypoxia.[22] To our knowledge, there has been no study either about the status of AOPPs in SH or about the effect of PT, and our study is the first to investigate the levels of AOPPs in newborns with SH requiring PT, as well as the effect of intensive PT on the levels of AOPPs. In comparison to healthy control newborns without SH, we detected no significant difference in AOPP levels in newborns with SH. However, with intensive PT, AOPP levels decreased significantly, and the decrease in AOPP levels was more prominent in cases with a more prominent bilirubin decrease (correlation analysis).

The present study has two limitations. The first is that studying jaundiced newborns with critically higher serum bilirubin levels (i.e., >25–30 mg/dL) might have given different and more significant results. The second is that following the parameters of oxidative stress in a more longitudinal manner would have revealed the normalization period of these parameters.

In the present study, S100B levels were significantly higher in newborns with SH than in those without hyperbilirubinemia, and these levels did not demonstrate a significant decrease after intensive PT. However, the normalization period of S100B levels in newborns with SH should be followed with further longitudinal studies. Accordingly, MDA levels, which were also higher before PT, decreased after intensive PT; however, whether this decrease is due to the decrease in serum bilirubin levels or due to the effect of intensive PT itself remains to be determined in further studies. Unlike S100B and MDA, AOPP levels were not significantly different in newborns with SH. However, the significant decrease in AOPP levels after PT confirms the antioxidant efficacy of intensive PT.

Conflicts of interest

The authors have no affiliations or financial involvement with any organization that has a financial interest in the issues studied in the present article.

Acknowledgments

We thank Professor Dr Mustafa Akcakus and Professor Dr Muhittin A. Serdar for their valuable contribution.

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