DOI: 10.24938/kutfd.1118250 Kırıkkale Üniversitesi Tıp Fakültesi Dergisi 2023;25(1):16-22
Original Article *Özgün Araştırma*

THE EFFECT OF PHOTOTHERAPY TREATMENT ON OXIDATIVE STRESS AND INFLAMMATORY RESPONSE IN NEWBORNS

Yenidoğanlarda Fototerapi Tedavisinin Oksidatif Stres ve İnflamatuar Yanıt Üzerine Etkisi

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ABSTRACT

Objective: A reason for oxidative stress is photodynamic reactions with phototherapy. It is thought that phototherapy changes cytokine production which leads to different effects on the immune system. The aim of this study is to investigate the effects of light-emitting diode (LED) phototherapy and conventional phototherapy on oxidative stress and inflammatory response in neonatal hyperbilirubinemia.

Material and Methods: Thirty-term newborns treated with phototherapy were enrolled on the study group. The study group was divided into LED phototherapy (n=15) and conventional phototherapy (n=15) subgroups. The control group consisted of 30-term healthy newborns. Blood samples were taken before phototherapy, at the 24th hours of phototherapy and after phototherapy treatment. Bilirubin, malonaldehyde (MDA), total oxidative stress (TOS), total antioxidant capacity (TAC), TNF-α, IL-6 and IL-8 levels were evaluated between groups.

Results: There were no significant differences in MDA and TAC in the conventional phototherapy group both before and after phototherapy. However, TOS levels were significantly higher during and after phototherapy than before phototherapy in the conventional phototherapy group. In the same group, IL-8 levels were significantly higher during phototherapy than after phototherapy levels. Whereas, there were no significant differences in oxidative stress and inflammatory response parameters in the LED phototherapy group.

Conclusion: While conventional phototherapy increases oxidative stress and acts at some stages of the inflammatory response, there are no effects of LED phototherapy on antioxidant defence systems and inflammatory response.

Keywords: *Hyperbilirubinemia, inflammatory response, phototherapy, oxidative stress*

ÖZ

Amaç: Oksidatif stresin bir nedeni fototerapi ile oluşan fotodinamik reaksiyonlardır. Fototerapinin bağışıklık sisteminde farklı etkilere yol açan sitokin üretimini değiştirdiği düşünülmektedir. Bu çalışmanın amacı, hiperbilirubinemik yenidoğanlarda ışık yayan diyot (LED) fototerapi ve konvansiyonel fototerapinin oksidatif stres ve inflamatuar yanıt üzerindeki etkilerini araştırmaktır.

Gereç ve Yöntemler: Fototerapi ile tedavi edilen 30 term yenidoğan çalışma grubuna alındı. Çalışma grubu LED fototerapi (n=15) ve konvansiyonel fototerapi (n=15) alt gruplarına ayrıldı. Kontrol grubu 30 günlük sağlıklı yenidoğanlardan oluşturuldu. Fototerapi öncesi, fototerapinin 24. saati ve fototerapi tedavisi sonrası kan örnekleri alındı. Gruplar arasında bilirubin, malonaldehit (MDA), toplam oksidatif stres (TOS), toplam antioksidan kapasite (TAC), TNF-α, IL-6 ve IL-8 düzeyleri değerlendirildi.

Bulgular: Konvansiyonel fototerapi grubunda fototerapi öncesi ve sonrası MDA ve TAC açısından anlamlı fark yoktu. Bununla birlikte, fototerapi sırasında ve sonrasında TOS seviyeleri, konvansiyonel fototerapi grubunda fototerapi öncesine göre anlamlı derecede yüksekti. Aynı grupta IL-8 seviyeleri fototerapi sırasında fototerapi sonrası ile karşılaştırıldığında anlamlı derecede yüksekti. LED fototerapi grubunda ise oksidatif stres ve inflamatuar yanıt parametrelerinde farklılık yoktu.

Sonuç: Konvansiyonel fototerapi oksidatif stresi artırırken ve inflamatuar yanıtın bazı aşamalarında etki gösterirken, LED fototerapinin antioksidan savunma sistemleri ve inflamatuar yanıt üzerinde herhangi bir etkisi gösterilememiştir.

Anahtar Kelimeler: *Hiperbilirubinemi, inflamatuar yanıt, fototerapi, oksidatif stres*

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Received / Geliş Tarihi: 26.07.2022 **Accepted** / Kabul Tarihi: 30.10.2022

KÜ Tıp Fak Derg 2023;25(1):16-22
Doi: 10.24938/kutfd.1118250

INTRODUCTION

Indirect hyperbilirubinemia is one of the most common reasons for hospitalization during the first weeks of life. 60% of term infants and 80% of preterm infants develop jaundice in the first week of life. Untreated hyperbilirubinemia is a major health problem that might cause acute bilirubin encephalopathy, mental retardation, kernicterus, and cerebral palsy (1).

Phototherapy is commonly used in the neonatal intensive care units for neonatal indirect hyperbilirubinemia treatment. It provides the formation of bilirubin isomers and photooxidation products by inducing rapid oxidation reactions (2). However, it is not treatment free of side effects. Photodynamic reactions during the oxidative stress in phototherapy may lead to lipid, protein and deoxyribonucleic acid damage (3). It is reported that oxidative stress and lipid peroxidation cause some changes in the inflammatory response that lead to cytokine production. It is also known inflammatory response and cytokine production play a role in many neonatal diseases such as bronchopulmonary dysplasia, periventricular leukomalacia, retinopathy of premature, necrotizing enterocolitis, patent ductus arteriosus (PDA) (4).

Although the side effects of light-emitting diode (LED) phototherapy are less than conventional phototherapy, there is insufficient data regarding the effects on oxidative stress and inflammatory cytokines.

Total oxidative stress (TOS) consists of radicals that can be taken directly from the outside or released during some reactions in the body. These radicals can cause genetic damage by affecting DNA, loss of function in cell membranes by affecting lipids, and loss of function in enzymes by affecting proteins. The total oxidant status provides information about the overall oxidation state of the body. In physiological conditions, the organism has a complex antioxidant defense system that defies free radicals and oxidative stress. The major contribution to the total antioxidant capacity (TAC) is provided by antioxidant molecules in the plasma (5). Malondialdehyde (MDA) is an end product of polyunsaturated fatty acids. It is often used in the evaluation of lipid peroxidation (6,7). Tumour necrosis factor-α (TNF-α), interleukin 6 (IL-6), and IL-8 are proinflammatory cytokines that play a role in regulating growth, cell activation, differentiation, and directing the immune cells to the sites of infection (8).

In this study, the effects of LED phototherapy and conventional phototherapy on oxidative stress and inflammatory response in neonatal hyperbilirubinemia were investigated.

MATERIALS AND METHODS

This prospective study included 60 newborns. At first, the patients were divided into two groups as indirect hyperbilirubinemia and control groups, and then they were divided into subgroups according to the phototherapy device used.

Thirty newborns with indirect hyperbilirubinemia were divided into two study groups: LED phototherapy (Group 1a) and conventional phototherapy (Group 1b).

The inclusion criteria of the study groups were;

1. Gestational age between 38 and 42 weeks, appropriate for gestational age (AGA).
2. Newborns who have no risk factor (Risk factors are maternal fever, premature rupture of membrane, maternal urinary system infection or vaginitis)
3. Requirement of phototherapy (American Academy of Pediatrics guideline was used for phototherapy threshold) (1).

Exclusion criteria were the history of fetal distress, asphyxia,sepsis and immune hemolytic anemia.

The control group (Group 2) consisted of 30 terms healthy AGA newborns that were at the same gestational and postnatal age.

The inclusion criteria of the control group were;

1. There is no history of maternal systemic disease, medicine use, maternal fever, premature rupture of membrane, fetal distress or asphyxia
2. There is no jaundice on physical examination
3. Lack of blood group incompatibility between mother and baby

A physical examination was performed by a pediatrician at the time of hospitalization. Demographic

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characteristics, clinical and laboratory data of newborns were recorded.

Conventional phototherapy was used by a standard phototherapy device (Philips TL 20W/52 Low pressure), consisting of 3 white and 3 blue fluorescent lamps, at a wavelength of 430-470 nm with an intensity of 20 µW/cm2/nm.

LED phototherapy was applied at a wavelength of 450-470 nm, at an intensity of 30 uW/cm²/nm by neoBLUE® LED Phototherapy device (Natus Medical Inc. San Carlos, CA, USA).

The newborns were put naked, except for diapers and eye patches, in an incubator or crib. The phototherapy device was placed 30 cm above the newborn.

Blood samples were taken from newborns in the study groups before phototherapy, at the 24th hour of phototherapy and after 24 hours of completing phototherapy. A blood sample was taken once from babies in the control group.

After 3 ml of blood sample was obtained by venipuncture, it was transferred to the laboratory immediately. Then it was centrifuged at 3000 rpm for 10 minutes. As soon as the plasma was separated, it was stored at −80°C for analysis. All the plasma samples were dissolved at room temperature and were analyzed for oxidant stress and inflammatory response. The oxidant stress was evaluated with TOS, TAC, and MDA. The inflammatory response was analysed with IL-6, Il-8, and TNF-α.

The study was approved by the ethics committee of the Faculty of Medicine, Kırıkkale University (2011/0056).

Statistics

Statistical analyses were performed using the statistical package SPSS for Windows V.16.0 (SPSS Inc, Chicago, Illinois, USA). Continuous variables are presented as the means±SD, while categorical variables are given as frequencies and percentages. For data not normally distributed median values and ranges are used. The Mann– Whitney U test and Wilcoxon Signed Ranks test were used to assess differences between the groups at the various time points. A p value of <0.05 was considered statistically significant.

RESULTS

There were 30 newborns in Group 1 (each subgroup has 15 newborns) and 30 newborns in Group 2.

There were no statistically significant differences between the groups in terms of gender, gestational, postnatal and maternal age. The demographic characteristics of the groups were shown in Table 1.

Table 1: Demographic characteristics of study and control groups

	Study group	**Control group**	***p***
Maternal age (year)*	28.1±6	28.2±5.2	*NS*
C/S, n (%)	15(50)	22(73.3)	*<0.05*
Gestational age (weeks)*	38.5±0.8	38.5±0.7	*NS*
Postnatal age (day)*	5.0±1.6	4.3±0.9	*NS*
Birth weight (g)*	3345±505	3193±518	*NS*
Male, n (%)	13(43)	16(53)	*NS*

*; mean±SD, NS; p>0.05

Before phototherapy, the mean bilirubin level was 18.5±1.6 mg/dl in the study group. The mean bilirubin level was 18.8±1.8 and 18.2+1.4 for infants treated with LED (Group 1a) and conventional phototherapy (Group 1b), respectively.

In the study groups (Group 1a and Group 1b), before phototherapy, the level of TAC and MDA were significantly higher than those of the control group (p=0.03, p=0.001, respectively). IL-6 and IL-8 levels were lower in the study groups compared to the control group (p=0.021, p=0.032). There was no statistically significant difference between the groups in terms of TOS and TNF-α (Table 2).

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Table 2: Evaluation of oxidative stress and inflammatory response levels before phototherapy treatment in study and control group

	Study group	Control group	*p*
IL-6	5.42	7.64	*0.021*
IL-8	17.79	26.01	*0.032*
TNF-α	4.81	4.26	*0.564*
MDA	1.94	1.65	*0.001*
TAC	0.28	0.19	*0.038*
TOS	31.72	35.52	*0,055*

MDA; Malondialdehide, TOS; Total oxidative stress, TAC; Total antioxidant capacity

In the conventional phototherapy group, the median TOS levels were significantly higher at 24 hours of phototherapy (p=0.01) and after phototherapy (p=0.033). The median IL-8 levels were significantly higher at 24 hours when compared to after phototherapy (p=0.012). There were no significant differences regarding the median serum level of MDA, TAC, IL-6 and TNF-α in the conventional phototherapy group before, at 24 hours and after phototherapy (Table 3).

When the LED phototherapy group was analyzed, no differences were found in the levels of MDA, TAC, TOS, IL-6, IL-8, and TNF-α before, during or after phototherapy (p>0.05) (Table 4).

DISCUSSION

In this study we found that the levels of TAC were significantly higher in the study groups than in the control group. It is known that there is an insufficient antioxidant defense against oxidative stress in the newborn period. Bilirubin is one of the molecules that contribute to the antioxidant defense system. Bulut et al reported that TAC was significantly higher in the neonatal hyperbilirubinemia group than in the control group (9).

MDA is a substance of lipid peroxidation induced by free radicals. In this study, we found that levels of MDA were significantly higher in neonatal hyperbilirubinemia groups than those in the control group. Similarly, Yigit et al reported that MDA levels were increased in hyperbilirubinemia. They also showed that correlation between MDA and bilirubin in newborns with hemolytic disease, however, no correlation was observed in babies with non-hemolytic jaundice (7). However, there was no correlation between bilirubin level and MDA in our study, the reason for that might be none of our patients had hemolytic disease.

When the effect of phototherapy on the antioxidant system was evaluated, in the conventional phototherapy group, the median TOS levels were significantly high during and after phototherapy. However, in the LED phototherapy group, there was no difference in the level of TOS before, during or after phototherapy.

Aycicek et al reported high levels of serum TOS and lipid hydroperoxide after exposure to phototherapy (10). Kale et al found that serum TAC levels were significantly decreased after conventional phototherapy with fluorescent lamps, LED phototherapy, and fiberoptic phototherapy (11). On the other hand, TOS increases markedly after conventional phototherapy and LED phototherapy but not after fiberoptic phototherapy. Allam et al reported that in both conventional and LED phototherapy oxidative stress indexes were increased in preterm infants after phototherapy. However, in the conventional phototherapy group, TAC was lower and TOS was higher as compared to the LED group (12).

In the first days of life, the newborn's immune response can be influenced by many factors such as birth stress, environmental factors, and postnatal age. The mode of delivery and medicine given to the mother during the birth are also reasons that affect the immune system of the newborn (13).

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Table 3. Evaluation of oxidative stress and inflammatory response levels before, during and after phototherapy in conventional phototherapy group

	Before phototherapy	24h of phototherapy	After phototherapy	p^{1-2}	p^{1-3}	P^{2-3}
IL-6	6.70	8.41	6.12	*0.730*	*0.268*	*0.221*
IL-8	17.48	20.57	14.61	*0.280*	*0.589*	*0.012*
TNF-α	3.37	2.70	3.12	*0.061*	*0.069*	*0.932*
MDA	1.76	1.61	1.47	*0.443*	*0.156*	*0.182*
TAC	0.23	0.27	0.42	*0.755*	*0.061*	*0.060*
TOS	29.19	32.87	32.80	*0.010*	*0.033*	*0.887*

MDA; Malondialdehide, TOS; Total oxidative stress, TAC; Total antioxidant capacity

Table 4. Evaluation of oxidative stress and inflammatory response levels before, during and after phototherapy in LED phototherapy group

	Before phototherapy	24h of phototherapy	After phototherapy	p^{1-2}	p^{1-3}	P^{2-3}
IL-6	4.98	4.65	4.31	*0.442*	*0.426*	*0.064*
IL-8	18.10	17.48	18.80	*0.820*	*0.532*	*0.233*
TNF-α	5.91	5.17	5.34	*0.116*	*0.125*	*0.910*
MDA	2.15	2.26	2.15	*0.307*	*0.650*	*0.755*
TAC	0.31	0.20	0.28	*0.733*	*0.925*	*0.410*
TOS	34.71	34.94	32.74	*0.514*	*0.410*	*0.191*

MDA; Malondialdehide, TOS; Total oxidative stress, TAC; Total antioxidant capacity

In our study, IL-6 and IL-8 levels were significantly higher in the control group than those in the study group, but there was no significant difference in TNF-α levels between the groups. Newborns who had fetal distress or asphyxia are excluded from our study and the postnatal ages of infants were similar in both groups. For this reason, high levels of IL-6 and IL-8 in the control group may be due to higher cesarean rates in the control group (73.3%) than in the study group (50%) and/or anaesthetic drugs applied during cesarean section.While oxidative stress-related inflammation plays an important role in the pathogenesis of many diseases, few studies were shown that photo-oxidation initiates inflammation (14). Some studies have shown that cytokine production can change with ultraviolet (UV) B rays, and also with phototherapy (15,16). After exposure to UV radiation, peripheral mononuclear cells and keratinocytes which are sources of cytokines such as TNF-α, IL-1, IL-3, IL-6, IL-8 and IL-10 are stimulated and the immune mediators are released (15). Kurt et al showed that after phototherapy IL-1β, IL-8 and TNF-α levels were increased but there was no significant change in the level of IL-6 (17).

In the conventional phototherapy group, IL-6 and IL-8 levels increased and TNF-α levels decreased at 24 hours of phototherapy when compared to those before phototherapy. In the LED phototherapy group, TNF-α, IL-6, and IL-8 levels decreased at 24 hours of phototherapy when compared to those before phototherapy. However, differences in both groups were not statistically significant, and this might be related to the limited number of patients. Yet, a significant decrease in IL-8 levels was observed only after 24 hours of completing conventional phototherapy ($p < 0.05$). It has been reported that serum IL-8 levels in patients with psoriasis decreased significantly after phototherapy, and IL-8 might be a sensitive biological biomarker for evaluating the effectiveness of psoriasis therapy (18).

Our study evaluates both LED and conventional phototherapy on oxidative stress and inflammatory response. Nevertheless, the small number of patients in the study groups is the limitation of the study. Therefore, our results need to be supported by a larger study group.

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In conclusion, while LED phototherapy may has no effect on the oxidant / antioxidant balance, conventional phototherapy has shifted this balance to the oxidative side. In addition, when the effect of LED phototherapy on the inflammatory response is not observed, conventional phototherapy had effects in some steps of inflammatory response.

Conflict of Interest: The author has no conflict of interest to declare.

Researchers' Contribution Rate Statement:

Concept/Design: DA,GA; Analysis/Interpretation: DA,GA,UK,OA; Data Collection: GA,OA,UK; Writer: NG; Critical Review: NG,DA; Approver : DA,GA,UK,OA,NG

Ethics Committee Approval: The ethics committee approval of the study was obtained from the Kırıkkale University Clinical Research Ethics Committee (Date:05.04.2011 / Decision no: 2011/0056)

*Funding***:** The study was carried out with the support of Kırıkkale University Scientific Research Projects Coordination Unit (Project number: 2011/48).

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