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Original article

Pro-oxidant /Antioxidant Balance in Neonatal Jaundice Pre and After Phototherapy

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Abstract

Background and Aim: This prospective observational study was designed aiming at evaluation of the possible effect of phototherapy on pro-oxidant/antioxidant balance (PAB) in newborns with jaundice. **Patients and Methods**: This study included forty newborns admitted to the NICU of Benha University Hospitals. The patients were full term, weighting > 2500 gm, aged between 2 and 14 days, with idiopathic unconjugated hyperbilirubinemia (bilirubin levels > 15 mg/dl) and were treated with phototherapy. All the studied patients were subjected to full medical history taking, complete clinical examination, and laboratory investigations as: complete blood count, ABO and Rh group, serum bilirubin total, direct and indirect, and PAB. Follow up investigations of serum total bilirubin (STB) levels, and PAB were recorded at the end of the phototherapy process. **Results:** The median pro-oxidant significantly increased after phototherapy (52.5 **nmol/L)** than before it (31.8 **nmol/L**) (P-value <0.001). In contrast, Antioxidant significantly declined after phototherapy (1.4 **nmol/L**) than before it (2.2 **nmol/L**) (P-value < 0.001). The median PAB significantly increased after phototherapy (38.3 **HK**) than before it (14.3 **HK**) (P-value <0.001). PAB percent increase following phototherapy showed a significant negative correlation with total bilirubin percent decrease following phototherapy. **Conclusion:** Decreased levels of bilirubin after phototherapy cause a shift in the PAB value in favor of oxidants.

Keywords: phototherapy; Pro-oxidant; antioxidant; PAB; Jaundice

Introduction

Neonatal jaundice is yellowish discoloration of the skin, conjunctiva, and sclera from elevated serum or plasma bilirubin in the newborn period (1)**.** About 60%–80% of healthy neonates are expected to present with idiopathic jaundice. The dermal icterus is first noted in the face and when the bilirubin level rises, it proceeds to the trunk then to the extremities. This condition is common in 50%–60% of newborns in the first week of life (2).

Recently, phototherapy has been proven to be associated with oxidative stress, lipid peroxidation and DNA damage (3)**.**

As for the treatment of severe hyperbilirubinemia, phototherapy is currently the most widely used form of therapy. However, reports have shown that oxidant/antioxidant balance is also disturbed during such practice, as some results indicated an increased oxidative stress index after phototherapy (4)**.**

This study aimed at evaluation of the possible effect of phototherapy on pro-oxidant/antioxidant balance (PAB) in newborns with Jaundice

Patients and methods

This prospective observational study was designed to evaluate the effect of phototherapy on pro-oxidant/antioxidant balance in newborns with Jaundice. The study was conducted in accordance with ethical principles that had their origin in the Declaration of Helsinki, and was approved by the Ethical Committee of Benha Faculty of Medicine (approval number: RC10–6-2020). Written informed consent was obtained from the parents of the neonates who were included in the study.

This study was conducted during the period from October 2020 to November 2021, and included forty newborns admitted to the NICU of Benha University Hospitals,

Inclusion criteria

This study was conducted on full term babies with weight $\geq$ 2500 gm, age 2-14 days, with idiopathic unconjugated hyperbilirubinemia (bilirubin levels > 15 mg/dl) and were treated with phototherapy which was performed based on principles defined by the American Academy of Pediatrics (AAP) (5); only interrupted by breastfeeding, changing of diapers, and taking blood samples.

Exclusion criteria

The neonates were excluded from the study if they had sepsis, birth asphyxia, respiratory distress syndrome (RDS), severe birth defects, maternal eclampsia or preeclampsia, ABO or Rh incompatibility, polycythemia,

G6PD, positive direct Coombs test or signs of jaundice during the first 24 hours after birth.

All the studied patients were subjected to full history taking, complete clinical examination and laboratory investigations as complete blood count, ABO and Rh group, serum bilirubin total and direct, urea and creatinine, and PAB. Follow up of serum total bilirubin (STB) levels, and PAB were recorded at the end of the phototherapy process.

Blood tests

Three ml's venous blood were collected from each subject under complete sterile aseptic condition in a sterile test tube. Serum was obtained by centrifugation of clotted samples at 3000 g for 10 minutes. All samples were coded and stored at −20°C, to detect the serum level of prooxidants and antioxidants by quantitative sandwich ELISA technique using ELISA kit for research (Cat #: E-02417hu 96T, Cloud-Clone Corp Co., Ltd, Katy, TX 77494, USA) and (Cat #: E-01738hu 96T, Cloud-Clone Corp Co., Ltd, Katy, TX 77494, USA).

Statistical methods

Data management and statistical analysis were done using SPSS version 25 (IBM, Armonk, New York, United States). Quantitative data were assessed for normality using the Shapiro-Wilk test and direct data visualization methods. According to normality testing, numerical data were presented as means and standard deviations or medians and ranges. Categorical data were presented as numbers and percentages. Quantitative data were compared before and after phototherapy using paired t-test or Wilcoxon signed ranks test for normally and non-normally distributed numerical variables, respectively. All statistical tests were two-sided. P values less than 0.05 were considered significant

Results

This study was conducted on 40 newborns admitted to the NICU of Benha University Hospitals with jaundice for phototherapy. The mean gestational age was 37 weeks. The mean neonatal age was seven days. About two-thirds of the neonates (62.5%) were males. The most frequent mode of delivery was cesarean section (67.5%). The mean birth weight was 2858 grams. Positive consanguinity was reported in 5% of the patients ***(Table1)***.

Total and indirect bilirubin significantly declined after phototherapy (8.9 and 8.2 **mg/dL**, respectively) compared to before phototherapy (17 and 15.9 **mg/dL**,

respectively) (P-value <0.001 for each) ***(Table 2).***

The median pro-oxidant significantly increased after phototherapy (52.5 **nmol/L**) compared to before it (31.8 **nmol/L**) (P-value <0.001). In contrast, Antioxidant significantly declined after phototherapy (1.4 **nmol/L**) compared to before it (2.2 **nmol/L**) (P-value < 0.001). The median PAB significantly increased after phototherapy (38.3 **HK**) compared to before it (14.3 **HK**) (P-value <0.001) ***(Table 3 & figure 1).***

The PAB percent increase following phototherapy showed a significant negative correlation with the total bilirubin percent decrease following phototherapy (r = -0.421, P-value = 0.008). ***(Table 4)***

The PAB percent increase following phototherapy did not show any significant change related to neonatal gender (P-value = 0.289), mode of delivery (P-value = 0.916), maternal diabetes mellitus (P-value = 0.198), maternal hypertension (P-value = 0.227), Premature rupture of membranes (PROM) (P-value = 0.955), or neonatal respiratory distress (P-value = 0.554) ***(Table 5).***

Table (1) General characteristics of the studied patients

General characteristics		
Gestational age (weeks)	Mean ±SD	37 ±2
Age (days)	Mean ±SD	7 ±1
Gender	Males n (%)	25 (62.5)
	Females n (%)	15 (37.5)
Mode of delivery	NVD n (%)	13 (32.5)
	CS n (%)	27 (67.5)
Birth weight (gm)	Mean ±SD	2858 ±173
+ve consanguinity	n (%)	2 (5.0)

NVD: Normal vaginal delivery CS: Cesarean section

Table (2) Total and direct bilirubin before and after phototherapy

Total Bilirubin	**Mean ±SD**	**P-value**
Before phototherapy (mg/dl**)**	17 ±1.1	**< 0.001**
After phototherapy (mg/dl**)**	8.9 ±1	
Direct Bilirubin	**Mean ±SD**	**P-value**
Before phototherapy (mg/dl**)**	1.1 ±0.4	**<0.001**
After phototherapy (mg/dl**)**	0.7 ±0.2	

Paired t-test was used

Table (3) Pro-oxidant, antioxidant, and PAB before and after phototherapy

			P-value
Pro-oxidant			
Before phototherapy (nmol/L)	Median (range)	31.8 (7.9 - 84.6)	**<0.001**
After phototherapy (nmol/L)	Median (range)	52.5 (22.4 - 112.6)	
Antioxidant			
Before phototherapy (nmol/L)	Mean ±SD	2.2 ±0.2	**< 0.001**
After phototherapy (nmol/L)	Mean ±SD	1.4 ±0.4	
PAB			
Before phototherapy (HK)	Median (range)	14.3 (3.1 - 45.7)	**<0.001**
After phototherapy (HK)	Median (range)	38.3 (13.9 - 89.1)	

Paired t-test was used for antioxidant. Wilcoxon signed ranks test was used for pro-oxidant and PAB
PAB: Pro-oxidant / antioxidant balance

Table (4) Correlation between PAB % increase after phototherapy and other parameters

Parameters	**PAB % increase**	
	r	**P-value**
Gestational age (weeks)	-0.287	0.076
Age (days)	0.172	0.296
Birth Weight (gm)	-0.158	0.337
Urea (mg/dl)	0.088	0.598
Creatinine (mg/dl)	-0.117	0.477
% decrease in total bilirubin	**-0.421**	**0.008**
% decrease in direct bilirubin	-0.191	0.245
Hemoglobin (gm/dl)	0.098	0.551
Hematocrit (%)	0.065	0.695
WBCs (× 10^9/L)	0.016	0.926
Platelet (×10^3/l)	-0.082	0.62
RBCs (× 10^{12}/L)	0.228	0.162

Spearman's correlation was used r: Correlation coefficient

Table (5) PAB % increase after phototherapy according to different parameters

parameters		PAB % increase	P-value
Neonatal gender	Males	134.5 (24.9 - 705.8)	0.289
	Females	222.4 (25.3 - 573.9)	
Mode of delivery	NVD	229.4 (24.9 - 573.9)	0.916
	CS	180.2 (25.3 - 705.8)	
Diabetes mellitus	Yes	112 (24.9 - 451.9)	0.198
	No	211 (25.3 - 705.8)	
Hypertension	Yes	224.2 (132.4 - 689.4)	0.227
	No	180.2 (24.9 - 705.8)	
PROM	Yes	184.4 (68.9 - 449.5)	0.955
	No	202 (24.9 - 705.8)	
Neonatal respiratory distress	Yes	134.5 (32.8 - 403.1)	0.554
	No	206.7 (24.9 - 705.8)	

Mann Whitney U test was used PROM: Premature rupture of membrane

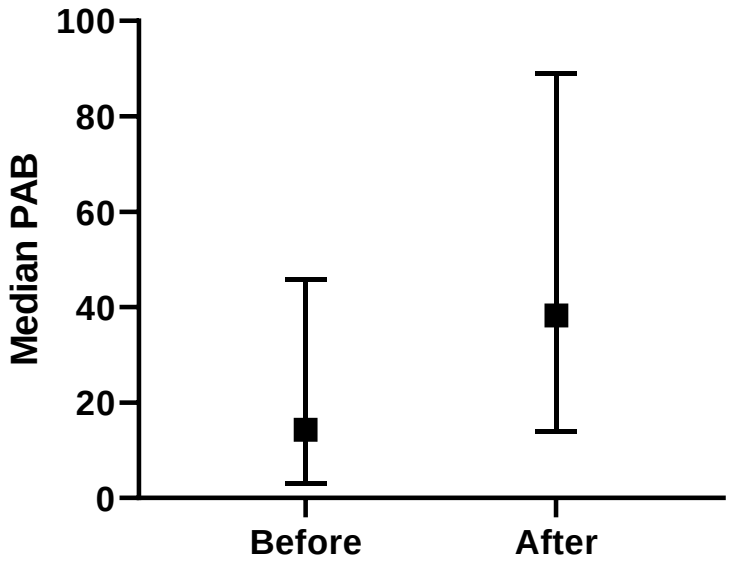

Figure (1): PAB before and after phototherapy

Discussion

In the present study, the median pro-oxidant significantly increased after phototherapy (52.5 nmol/L) compared to it before phototherapy (31.8 nmol/L) (P-value <0.001). The median antioxidant significantly declined after phototherapy (1.4 nmol/L) compared to it before phototherapy (2.2 nmol/L) (P-value <0.001). The median PAB significantly increased after phototherapy (38.3 HK) compared to it before phototherapy (14.3 HK) (P-value <0.001).

In the research studying the impact of phototherapy on oxidative stress indices in preterm neonates with unconjugated hyperbilirubinemia it was reported that total oxidant status (TOS) and oxidative stress index (OSI) were significantly higher following phototherapy than before phototherapy. The total antioxidant capacity (TAC) was significantly lower after phototherapy than before it (6).

The effect of phototherapy on pro-oxidant/antioxidant balance in 70 icteric term neonates was studied. The average and standard deviation of bilirubin levels and PAB values were 18.90±2.97 HK and 16.29±9.83 HK respectively before phototherapy and 15.71±3.16 HK and 29.63±12.56 HK respectively during phototherapy and 12.37 ±3.57 HK and 40.91 ±13.35 HK respectively after phototherapy (7). The results of this study demonstrate that decreased levels of bilirubin after phototherapy caused a shift in the PAB value in favor of oxidants.

However, a study was conducted on 20 term and 16 preterm icteric neonates who needed 72 hours of continuous phototherapy to examine the antioxidant defense system in neonates undergoing phototherapy (8). To investigate for the possible incidence of oxidative stress as a result of phototherapy, the authors measured the serum levels of vitamin E and red blood cell antioxidant enzymes activities (superoxide dismutase, catalase, and glutathione peroxidase) before and after 72 hours phototherapy. The results showed no significant change in serum levels of vitamin E before and after 72 hours phototherapy. Accordingly, they did not confirm that phototherapy creates oxidative stress. However, it must be considered that the sample size was small and that a limited number of antioxidants were examined in this study.

Oxidative stress can be defined as an imbalance between the amount of reactive oxygen species (ROS) and the body’s ability to detoxify those operating defense systems.

This imbalance leads to damage to all the molecules in the cell, such as proteins, lipids and even the DNA, and interferes with the cellular signaling system. Bilirubin absorbs blue light in the range of 460-490 nm. Various tools working with different wavelengths and light intensity are now available for phototherapy (9)**.**

Phototherapy changes unconjugated bilirubin into oxidized bilirubin and its structural isomers that can be easily excreted in the stool and urine. It has been suggested that phototherapy has a negative effect on the oxidant/antioxidant defense system, leading to increased levels of oxidative stress in neonates undergoing phototherapy. Phototherapy may be associated with high levels of oxidative stress, rates of lipid peroxidation and damages to DNA (7)**.**

A comparison was performed between the Total Oxidant Status (TOS) and the antioxidant status in unconjugated neonatal hyperbilirubinemia before and after traditional phototherapy and LED light treatment. The conclusion was that increased TOS occurs after traditional phototherapy, but not after LED light treatment. Moreover, they it was reported that the Oxidative Stress Index was significantly higher in traditional phototherapy, compared to LED light treatment ($p<0.05$) (10).

Another study was done exclusively on preterm neonates, to compare antioxidant/oxidant parameters following conventional and LED phototherapy. It revealed that both conventional and LED phototherapy resulted in increased oxidative stress index. However, derangement of antioxidant-oxidant parameters was more relevant after conventional than LED phototherapy. It seems that even the type of lamp used in phototherapy can affect the outcome (11).

In the current study, PAB percent increase following phototherapy showed a significant negative correlation with the total bilirubin percent decrease following phototherapy ($r = -0.421$, P-value = 0.008) while there was no significant correlation between PAB percent increase and decrease in direct bilirubin, chronological age, birth weight, blood urea, serum creatinine as well as The CBC results (hemoglobin, RBCs hematocrit, WBCs, or platelets). Also, PAB percent increase following phototherapy did not show any significant difference according to neonatal gender (P-value = 0.289), mode of delivery (P-value = 0.916), neonatal respiratory distress (P-value = 0.554), maternal diabetes mellitus (P-value = 0.198), maternal hypertension (P-value = 0.227) or PROM (P-value = 0.955).

The relationships between bilirubin and oxidant/antioxidant status were evaluated in different clinical studies in term and preterm neonates. The preoxidant effects of bilirubin was attributed to many different mechanisms such as overstimulation of glutamate receptors; increased proinflammatory cytokines and activity of neuronal nitric oxide synthase (12).

Some clinical trials showed the antioxidant effect of bilirubin. These studies found that the high levels of bilirubin did not increase the oxidative stress and lipid peroxidation unless additional risk factors such as low albumin level, glucose-6-phosphate dehydrogenase deficiency, and hepatic glucuronyl transferase deficiency were presented (13).

Bilirubin, a power antioxidant, also can act as a powerful but silent neurotoxin. Although several attempts were made to determine the role of bilirubin in the oxidative/antioxidant balance, none of them reached a definitive consensus to determine whether bilirubin has an antioxidant capacity or causes oxidative stress leading to encephalopathy (4).

Our results were matched with a study which reported a statistically significant negative correlation only between the amount of bilirubin decrease with phototherapy and the advanced oxidation protein products (AOPPs) levels (14).

Significant positive correlations were found between total serum bilirubin (TSB) and both TOS and OSI, and a significant negative correlation was also reported between the TSB and the TAC. Also this study revealed a significant increase in TOS and OSI and a significant decrease in TAC after phototherapy treatment (6).

Our study is comparable also with the study done before (15) where it was reported that an increase in the oxidative stress after phototherapy exists and explained it by both the decrease in bilirubin and effect of phototherapy. Also, it was shown that hyperbilirubinemia and phototherapy have negative effects on oxidant/antioxidant defense system; leading to increased levels of oxidative stress in neonates underwent phototherapy treatment (7).

However, it was reported that high bilirubin level was shown to be a reason of oxidative stress (16 & 17). In another study it was reported that after phototherapy, serum TOS and OSI levels significantly increased whereas the serum level of TAC did not change significantly, DNA is highly sensitive for oxidative damage (18). Both conventional and intensive phototherapies were found to increase DNA damage in

mononuclear leukocytes in jaundiced term neonates. A non-significant relation between the total serum bilirubin and TOS and TAC, was proved (19). This may have been due to different duration of phototherapy, 12 hrs in their study and >48 hr in our study.

A recent systematic review (20), showed that studies in term infants give contradictory results, while studies in preterm infants suggest that the total serum bilirubin (TSB) increase is associated with an oxidative stress increase due to concurrent factors other than bilirubin level, such as heme oxygenase (HO) activity. Moreover, it could be speculated that low physiologic TSB values are associated with antioxidant effects, while high pathologic TSB values are associated with pro-oxidant effects.

Conclusion

Our study showed that the median pro-oxidant significantly increased after phototherapy while the median antioxidant significantly declined after phototherapy. The median PAB significantly increased after phototherapy. PAB percent increase following phototherapy showed a significant negative correlation with total bilirubin percent decrease following phototherapy. Therefore, we can conclude that decreased levels of bilirubin after phototherapy causes a shift in the PAB value in favor of oxidants.

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