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Impact of Phototherapy on Oxidative Stress Indices in Preterm Neonates with Unconjugated Hyperbilirubinemia

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ABSTRACT

Background: Neonatal jaundice is one of the most common neonatal problems especially in preterm ones. Preterm babies are treated with different types of phototherapy. Preterm neonates have a weak antioxidant defense mechanism. Both the hyperbilirubinemia and phototherapy have an impact on this antioxidant defense system.

Objective: In the current study we aimed to assess the effect of hyperbilirubinemia and phototherapy in the oxidant/antioxidant status of preterm newborns.

Subjects and Methods: Thirty late preterm (≥35 weeks) neonates with non-hemolytic neonatal jaundice in the 3-9 days of life were involved in the preset study, and received phototherapy. 15 healthy neonates of the same gestational and postnatal age without jaundice were included as a control. We assessed the total oxidant status (TOS), total antioxidant capacity (TAC), and oxidative stress index (OSI).

Results: TOS and OSI were significantly higher (P<0.01); TAC level was significantly lower (P<0. 01); in jaundiced preterm neonates than in control group. Following phototherapy there were significant increase in TOS and OSI (P<0. 01) and decrease in TAC (P<0.001).

Conclusions: Both hyperbilirubinemia and phototherapy induce oxidative stress in preterm neonates. **Recommendations**: preterm neonates with significant hyperbilirubinemia benefits of phototherapy overwhelm the risks.

Keywords: Preterms, oxidants, antioxidants, hyperbilirubinemia, oxidative stress

INTRODUCTION

Most preterm babies less than 35 weeks gestational age (GA) has hyperbilirubinemia, that present with jaundice. When these babies left untreated, an elevated bilirubin level (hyperbilirubinemia) can be symptomatic in the form of neurologic manifestations. Acute bilirubin encephalopathy (ABE) is an acute, progressive, and mostly reversible with aggressive intervention, however kernicterus (or chronic bilirubin encephalopathy [CBE]) is the syndrome of chronic, post-icteric and permanent neurologic sequelae that is associated with more serious condition and manifestations are usually irreversible [(1)]. Actually, increase in bilirubin production in preterm neonates adds to the risk of long-term neurodevelopmental impairment (NDI) or mortality due to neurotoxic effect of bilirubin [(2)] that can be manifested as the syndrome of bilirubin-induced neurologic dysfunction (BIND) [(3)]. Additionally, the incidence of neurologic damage in preterm infants is also low, so the risk-benefit spectrum for interventions should include a weight of the risk of overtreatment and the reduction of long-term post-icteric sequelae[(4)].

The increase of the production of endogenous and exogenous reactive oxygen in the human body is regulated by antioxidant defense systems [(5)]. However; these systems have not adequately protective effects on oxidative stress in neonates especially in the preterms. Lipid peroxidation and protein oxidation occur as result of insufficient antioxidant effects [(6)]. Reactive oxygen products can cause many serious neonatal diseases such as retinopathy of prematurity, bronchopulmonary dysplasia, periventricular l

eukomalacia, intraventricular hemorrhage, sepsis, necrotizing enterocolitis, and hypoxic ischemic

encephalopathy[(7)]. Nowadays, the effects of newborn diseases on the oxidative system have been

investigated by using different oxidative markers. There are several studies that reported the effects of both hyperbilirubinemia and phototherapy used for its treatment on the oxidative balance of neonates [(8)]. Some studies conclude that bilirubin has anti-oxidant effects in neonates [(9)]. Whereas, there are many clinical studies that demonstrate that bilirubin has prooxidant effects or no effect on oxidative balance of neonates [(10)]. Moreover, some clinical trials reported increased oxidative stress markers following phototherapy [(11)].

Oxidative stress most often occurs as a result of an imbalance between oxidants, antioxidants, and free radical production [(12)]. Moreover, preterm neonates have an immature antioxidant system which make them more vulnerable to oxidative stress.

Mildly elevated level of bilirubin may be associated with less morbidity and related mortality, which could be caused by the antioxidant properties of bilirubin. Bilirubin regarded as a potentially cytotoxic, lipid-soluble waste product, is now known to present potent antioxidant criteria preventing the oxidative

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damage induced by a wide range of oxidative stressors[4].

It is now known that conventional phototherapy given with fluorescent lamps have a negative impact on oxidant/antioxidant defense system and leads to increased oxidative stress in hyperbilirubinemic neonates [3].

Hence, understanding the effects of hyperbilirubinemia and phototherapy on oxidative balance becomes more complicated.

AIM OF WORK

Our aim was to assess the effects of hyperbilirubinemia and phototherapy on the global oxidant/antioxidant status in preterm neonates.

SUBJECTS AND METHOD

Late-preterm (≥35 weeks) newborn hospitalized for significant indirect hyperbilirubinemia requiring phototherapy in the 3-9 days of life was included.

Ethical approval and written informed consent:

An approval of the study was obtained from Al-Azhar University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

The current study included 45 late preterm newborn infant, they were classified into 2 groups, control group (G1) included 15 healthy preterm neonates without neonatal jaundice, and patient group included 30 healthy preterm infants with neonatal jaundice. The patient group before phototherapy was classified as group 2 (G2) and those following phototherapy as group 3 (G3).

Level of total serum bilirubin, total oxidant status (TOS), total antioxidant capacity (TAC) and the oxidative stress index (OSI) were measured in the control group and the patient groups at day 3. Also these parameters were measured in the patient group 48 hours after phototherapy.

All neonates were otherwise healthy with appropriate birth weights for gestational age (estimated by the last menstrual period and confirmed by ultrasound scan) and had no pathologic etiological factors for hyperbilirubinemia. Neonates with severe congenital malformations, maternal diabetes, maternal eclampsia–preeclampsia, birth asphyxia, respiratory distress even mild or transient, sepsis, hemolytic type of hyperbilirubinemia, ABO blood group or Rh incompatibility, positive direct Coombs test and those who were jaundiced within the first 24 h after birth were excluded from the study.

Criteria for starting phototherapy were based on the criteria defined by the American Academy of Pediatrics. For conventional phototherapy the IC-100 Phototherapy System (ErtunçÖzcan, Ankara, Turkey) consisting of four blue fluorescent lamps (OsramL 18W/67 Lumilux Blue, Germany, intensity: 10–15 μW/cm^2/nm, spectrum 430–470 nm).

During phototherapy all infants were kept completely unclothed with their eyes and genital regions covered. Phototherapy units were placed over the incubators 25–30 cm away from the infants.

Phototherapy was interrupted only for feeding, cleaning, and blood sampling. Gestational age, sex, birth weight, age at phototherapy, serum total bilirubin (STB) level at initiation and termination of phototherapy were recorded. Venous blood sampling (2 mL) was taken from a peripheral vein prior to and 48 hour after phototherapy to determine total antioxidant and oxidant capacity. Samples were centrifuged at 1500 ×g for 10 min within 30 min of collection, stored at −80 °C until analysis. As the measurement of different oxidants and antioxidants separately is not practical, and their oxidant and antioxidant effects are additive, the total antioxidant capacity (TAC) and total oxidant status (TOS) were measured using a reliable and sensitive direct measurement method[13].

Total antioxidant capacity 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 mmolTroloxequiv/L. Total oxidant status 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 μmol H_2O_2/L. The main components of serum TOS were hydrogen peroxide and lipid hydroperoxide[13]. Erel's TAC and TOS methods are colorimetric and automated and the precision of this assay is less than 3%. The TOS to TAC ratio was used as the oxidative stress index (OSI). The OSI value was calculated as follows: OSI = [(TOS) / (TAC) / 100] [14].

Statistical analyses

Collected data were coded and analyzed using Statistical Package for the Social Sciences (SPSS, version 15, Chicago, Illinois, USA). Continuous variables are presented as mean ± SD and Student's t-test was used for two-group comparisons of the normally distributed variables. The chi-square test was used to compare categorical variables. Correlation analyses were performed by the Pearson correlation test. Statistical significance was accepted at p-value $\leq$ 0.05.

RESULTS

In our current study there were no significant difference between the control group and the hyperbilirubinemia group regarding the clinical and demographic data (Table 1).

Table (1): Clinical and demographic data of the control and patient groups

Variable	Control group	Patient group	P value
Body weight (gm)	2300±270	2165±235	>0.05
Mean age (days)	6±3	5±3	>0.05
Gestational age (week)	35.6±1.2	35.2±1.4	>0.05
Sex (F/M)	9/6	18/12	>0.05
C.S (n)	9 (60%)	20 (66%)	>0.05
Length (cm)	42.5±7	41.9±6	>0.05
Head circumference (cm)	31.7±0.8	31.0±0.7	>0.05

The mean total serum bilirubin level, the total oxidant status (TOS) and the oxidative stress index (OSI) were significantly higher in the patient group than that of the control group. The total antioxidant capacity (TAC) was significantly lower in patient group before phototherapy than the control group (Table 2).

Table (2): Mean ±SD of total serum bilirubin (TSB), total oxidant status (TOS), total antioxidant capacity (TAC), and oxidative stress index (OSI) in both control and patient groups before phototherapy

Variable	Control group (G1) N=15	Patient group before phototherapy (G2) N=30	P value
Total serum bilirubin (TSB)(mg/dl)	3.2±0.8	16.15±3.35	0.001
Total oxidant status (TOS) (µmol H_2O_2 equiv./L)	9.8±2.1	11.92±3.297	>0.05
Total antioxidant capacity (TAC) (mmoltrolox equiv./L)	0.96±0.06	0.77±0.134	0.001
Oxidative stress index (OSI)	0.1±0.02	0.15±0.05	0.01

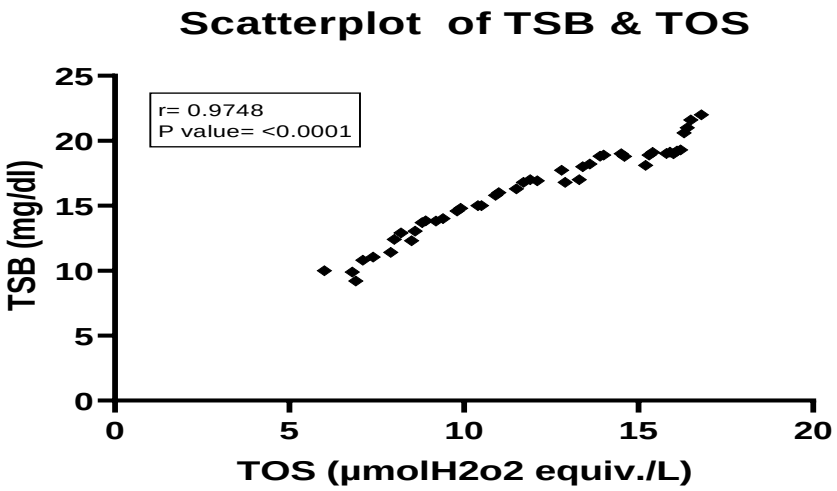

Fig. (1): Correlation between TOS and TSB in patients before phototherapy (r=0.97 P- value =0.001).

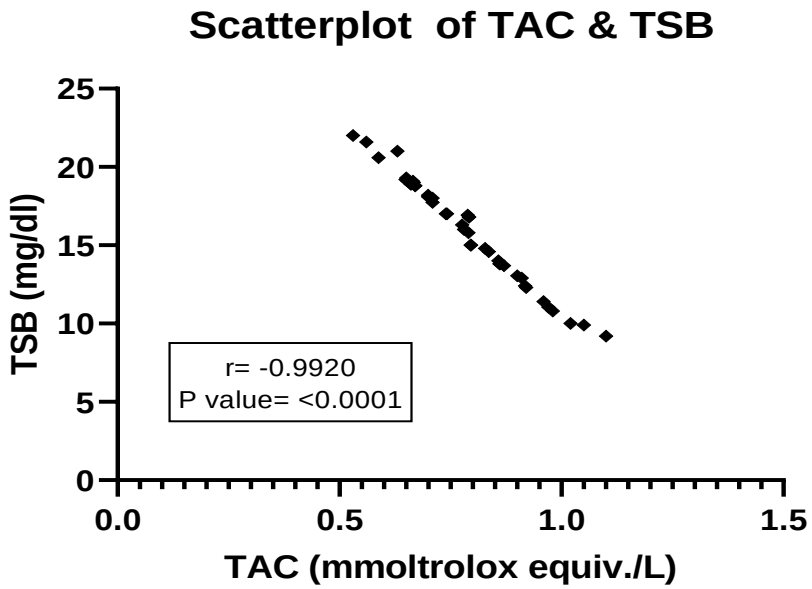

Fig. (2): Correlation between TAC and TSB in patients before phototherapy (r=-0.99 p-Value =0.001)

The TOS and OSI were significantly higher in patients following phototherapy than the patients before phototherapy. The TAC was significantly lower in the patients after phototherapy than before phototherapy (Table 3).

Table (3): Mean ±SD of total oxidant status (TOS), total antioxidant capacity (TAC), and oxidative stress index (OSI) in both control and patient groups before and after phototherapy

Variable	Control group (G1) N=15	Patient group before phototherapy (G2) N=30	Patient group after phototherapy (G3)N=30	P value		
				G1 VS G2	G1 VS G3	G2 VS G3
Total oxidant status (TOS) (µmolH2o2 equiv./L)	9.8±2.1	11.92±3.297	16.2±3.3	0.01	0.01	0.01
Total antioxidant capacity (TAC) (mmoltrolox equiv./L)	0.96±0.06	0.77±0.134	0.48±0.02	0.01	0.01	0.01
Oxidative stress index (OSI)	0.1±0.02	0.15±0.05	0.33±0.04	0.01	0.01	0.01

DISCUSSION

Bilirubin is a compound, which is known to be toxic to the central nervous system, and oxidative stress develops as a relevant event in the mechanism of bilirubin associated encephalopathy. However, more recent studies have showed that bilirubin may also have potent antioxidant properties. This was reinforced by the notion that serum bilirubin level increases in response to initial oxidative stress and its role as an important scavenger of reactive oxygen species was well documented. **Dogan *et al.*[(15)]** had suggested that the relationship between total serum bilirubin and antioxidant/oxidative stress can be presented by a quadratic correlation curve. So that, the role of bilirubin in the antioxidant panel and the oxidative stress parameter remained a conflicting issue to be further explored. As for the treatment of severe hyperbilirubinemia, phototherapy is currently the most widely used method for treatment of hyperbilirubinemia. However, reports showed that oxidant/antioxidant balance is also disturbed during such practice, as some results indicated an increased oxidative stress index after phototherapy [(16)]. Thus, the role of phototherapy in the oxidant/antioxidant status still considered an important issue to be further discussed.

The relationships between bilirubin and serum oxidant/antioxidant status were evaluated in different clinical studies in term neonates [3]. Few studies were carried on preterm neonates. In some of such studies, high levels of bilirubin were shown as a cause of oxidative stress[10]. 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[17]. Despite, some clinical trials showed the antioxidant effect of bilirubin, these clinical studies found that the high levels of bilirubin did not increase oxidative stress and lipid peroxidation unless additional risk such as low albumin level, glucose-6-phosphate dehydrogenase deficiency, and hepatic glucuronyltransferase deficiency were not presented[18]. Bilirubin, a power antioxidant, also can act as a powerful but silent neurotoxin at the most vulnerable stages of preterm period [4]**.** 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 [19]**.**

The results of a study done by **Sarici *et al.*** [20] are in favor of a positive correlation between hyperbilirubinemia and oxidative stress. Total serum bilirubin levels greater than 10 mg/dl are found to be hazardous to chromosomes [21]**.** Serum level of bilirubin is an antioxidant at <6 mg/dl and it will also act like an oxidative stress inducer at higher levels [22]**.**

The present study found significant positive correlation between TSB and both TOS and OSI, and a significant negative correlation between the TSB and the TAC. Also this study reveals a significant increase in TOS and OSI and a significant decrease in TAC after phototherapy treatment.

The results of our study were comparable to the study done by **Erol *et al.*** [23] who revealed an increase in oxidative stress after phototherapy and explained it by both the decrease in bilirubin and effect of phototherapy. Also **Boskabadi *et al.*** [24] showed that hyperbilirubinemia and phototherapy has negative effect on oxidant/antioxidant defense system, leading to increased levels of oxidative stress in neonates underwent phototherapy treatment.

Allam *et al.* [25] study was done exclusively among preterm neonates, to compare antioxidant-oxidant parameters following conventional and LED phototherapy; 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.

Thiagarajan et al. 2014 [26] **and Basu *et al.*** [10] reported that high bilirubin level was shown as a reason of oxidative stress. **Davutoglu *et al.*** [27] found an increase in the concentration of serum bilirubin parallel to an increase in oxidants and decrease in antioxidant activities.

Shekeeb Shahab *et al.* [18] had reported that phototherapy positively affect TOS and OSI but negatively affect TAC. **Vreman *et al.*** [28] stated that the exposure of neonates to phototherapy in the presence of sensitizer (bilirubin) resulted in oxidative injury to the red cell membrane presented by a significant increase in concentration of products of lipid peroxidation in the membrane and hemolysis. **Gathwala and Sharma** [29] showed that phototherapy induces oxidative stress in preterm neonates.

There were a number of studies which measured serum malondehyde (MDA) levels, which was an indicator of oxidative stresses such as **Putra *et al.*** [12] who found a significant increase in mean MDA level from before to after phototherapy treatment. Also **Surapaneni *et al*.**[30] had observed that there was a statistically significant increase in MDA levels (indicator of oxidative stress) in neonatal jaundice compared to controls. **Ozture *et al.*** [31] in their study found that plasma concentrations of MDA in neonates with non-hemolytic jaundice were significantly higher than those in healthy neonates. **Dahiya *et al.*** [32] in their study, demonstrated that both jaundice and phototherapy increased the oxidative stress (MDA were elevated significantly).

In contrast to the results of our study, The study done by **Erol *et al.*** [23] supported the antioxidant effect of bilirubin on newborn. **Kale *et al.*** [16] study revealed weak but significant correlation between the decline in TSB and the decrease in TAC, nevertheless, decrease in TAC cannot be caused by decrease in antioxidant bilirubin alone which had only small contribution (2.4%) to TAC**.** They also showed that phototherapy given to decrease STB levels led to lower TAC levels whatever of the type of the source and intensity of the light. Serum TOS was increased following conventional phototherapy with blue fluorescent lights and intensive LED phototherapy. Finally OSI increased in all cases after 24 h of phototherapy. **Gazzin *et al.*** [33] had stated that toxic products of oxygen and free radicals are minimized by bilirubin. **Kale *et al.*** [16] reported that the increase in the level of TOS seen after conventional phototherapy was not observed after light emitting diodes (LED) phototherapy. **Aycicek *et al.*** [14] reported that after phototherapy, serum TOS and OSI levels were significantly higher whereas serum level of TAC did not change significantly. DNA is highly sensitive for oxidative damage. Both conventional and intensive phototherapies were found to increase DNA damage in mononuclear leukocytes in jaundiced term neonates. **Dani and Colleagues** [34] found no significant relation between total serum bilirubin and TOS and TAC.

Abdel Latief *et al.* [35] observed significant decrease in MDA levels following phototherapy for 12 hours, in contrast to our results. This may have been due

to different duration of phototherapy, 12 hrs in their study and 48 hr in the current study. The decrease in MDA levels was likely occurred due to improvement in the condition of hyperbilirubinemia and new oxidative stress occurring after increased administration of phototherapy. **Shekeeb Shahab *et al.* [18]** had found that total bilirubin up to 20 mg/dl in term neonates had a positive correlation with TAC and a negative correlation with malondialdehyde (MDA) an important marker of lipid peroxidation. **Ozture *et al.* [31]** in their study showed that plasma MDA concentrations were significantly lower after phototherapy than before it, and no significant correlation was found between plasma MDA and plasma bilirubin before and after phototherapy than those in control.

CONCLUSIONS

Both hyperbilirubinemia and phototherapy induce oxidative stress in preterm neonates.

RECOMMENDATIONS

Preterm neonates with significant hyperbilirubinemia benefits of phototherapy overwhelm the risks. Despite that, phototherapy for prophylaxis of indirect hyperbilirubinemia should be restricted and cautiously used in association with antioxidant supplementation especially in neonates who have additional risk factors for ROS related diseases such as prematurity.

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