ANTIOXIDANT STATUS IN NEONATAL JAUNDICE BEFORE AND AFTER PHOTOTHERAPY

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ABSTRACT

Phototherapy has been related to increased oxidative stress and lipid peroxidation. In the present study, thirty full term jaundiced neonates with appropriate weight were analyzed before and after completion of phototherapy for malondialdehyde (MDA), reduced glutathione (GSH), total thiols, vitamin C and superoxide dismutase (SOD) levels in hemolysate and albumin levels in plasma. These parameters were analyzed in cord blood samples of 20 healthy neonates as control. It was observed that levels of MDA were elevated significantly ($p < 0.001$) in patients as compared to controls and that the levels increased significantly after phototherapy ($p < 0.001$). Levels of SOD were also found to be increased significantly as compared to controls and the levels rose after phototherapy ($p < 0.001$). On the other hand, the levels of non-enzymatic antioxidants such as GSH, total thiols and vitamin C were significantly low ($p < 0.001$) as compared to controls and the levels decreased significantly after phototherapy ($p < 0.001$). The plasma albumin levels also were found to be decreased significantly after phototherapy ($p < 0.01$). Therefore, phototherapy increases oxidative stress and should be used with care.

KEY WORDS

Neonatal Jaundice, Oxidative stress, Phototherapy.

INTRODUCTION

Neonatal jaundice is a common condition and phototherapy is an accepted modality for its management. It converts unconjugated bilirubin to its oxidation products as well as to photo and structural isomers, which are easily eliminated through gastrointestinal tract or lost in urine (1).

It has recently been demonstrated that phototherapy is an oxidative stress and can cause lipid peroxidation. Antioxidant activity in the serum of term neonates is lower than that of adults. It is still lower in preterm and low birth weight babies as compared to term babies (2, 3). Red blood cells are extremely susceptible to lipid peroxidation since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transitional metal catalysts. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro-oxidant potential (4).

Bilirubin is a sensitizer of singlet oxygen production. It behaves as an antioxidant especially the albumin bound fraction; therefore bilirubin-albumin ratio is important in deciding its activity. Red cell bilirubin acts as photo-sensitizer in the presence of phototherapy and brings about oxidative damage (5, 6).

The present study was, therefore undertaken to assess the susceptibility of red cells to oxidative damage by measuring red cell MDA and status of antioxidants by estimating reduced glutathione, total thiols, vitamin C, superoxide dismutase and plasma albumin.

MATERIALS AND METHODS

The study was carried out on 30 full term jaundiced neonates with appropriate weight, delivered normally in the labour room of Pt. B.D. Sharma PGIMS, Rohtak. These babies received continuous phototherapy except during feeding, cleaning and sampling. It was ensured that the jaundice was non-hemolytic with help of relevant investigations. Bilirubin levels were monitored for all babies at 24 hours interval. All the parameters were estimated before and after phototherapy. These were also assessed in cord blood samples of 20 neonates as control that did not develop jaundice in the neonatal period.

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Three ml. of venous blood was collected aseptically and hemolysate was prepared (7). Hemoglobin level of this hemolysate was estimated by Sahli's method and diluted to 1g% using distilled water, which was subsequently used for the following estimations:

MDA was estimated using the principle that lipid products react with thiobarbituric acid to give a red chromogen whose absorbance was read at 548nm spectrophotometrically (8).

Total thiols were estimated by Ellman reaction in which 5-5'dithiobis-2-nitrobenzoic acid reacts with total sulphhydryl groups and measured spectrophotometrically at 420 nm (9).

Reduced Glutathione was analysed using the method of Beutler *et al.* (10).

Vitamin C was determined using the property of its oxidation to dehydroascorbic acid in presence of Cu^{2+} , which then reacts with 2,4 dinitrophenylhydrazine to form a red bis-hydrazone having absorbance maxima at 529 nm (11).

SOD levels were estimated in the hemolysate by the method of Misra and Fridovich based on inhibition of auto-oxidation of epinephrine to adrenochrome at pH 10.2 (12).

Albumin was determined in plasma using the dye binding method of Doumas & Biggs (13).

Statistical analysis was done by applying unpaired Student 't' test between the control group and the patients before phototherapy. Paired Student 't' test was applied for comparison of the estimations before and after phototherapy.

RESULTS AND DISCUSSION

All the 30 patients in the study group received phototherapy ranging from 48 to 96 hours and majority

received it for 72 hours or more. The levels of bilirubin and albumin in plasma and their ratio when compared between controls and in patients before and after phototherapy are given in Table 1. The red cell levels of MDA (indicator of oxidative stress) and antioxidants like reduced glutathione, total thiols, vitamin C and SOD in three study groups are given in Table 2.

The results of the study show that the levels of MDA rose significantly from a level of 4.62 ± 0.52 nM/gHb to 5.63 ± 0.72 nM/gHb after treatment of neonates with phototherapy ($p < 0.001$). Rise in MDA could be due to increased generation of ROS (reactive oxygen species) as a result of phototherapy. Since bilirubin acts as a photo-sensitizer and becomes energized by phototherapy. The energy, thus gained, is subsequently transferred to molecular oxygen, thereby generating singlet oxygen and other ROS. These oxygen species, in turn, can oxidize many other important biomolecules including membrane lipids as well as bilirubin itself. As free bilirubin is mainly responsible for this, the ratio of bilirubin to albumin is important (5). Ostrea *et al.* (1985) studied cord blood cells, which were exposed to light in the presence of bilirubin and resulted in a significant ($p < 0.02$) increase in concentration of TBA reactants, diene conjugation and hemolysis (14).

In the present study, the red cell GSH levels decreased to 11.72 ± 2.86 M/gHb from 14.83 ± 2.75 M/gHb after phototherapy ($p < 0.001$) and similarly total thiols decreased to 43.04 ± 6.27 M/gHb from a pre phototherapy level of 47.46 ± 10.22 M/gHb ($p < 0.001$). It has been reported in literature that jaundice produces an oxidative stress as demonstrated by decreased levels of cellular glutathione, glutathione peroxidase and SOD in various studies (15, 16). Antioxidant status in jaundiced neonates after phototherapy is not clear. On searching the literature, we did not find any reports regarding the same except one report where Akisu *et*

Table 1. Plasma Bilirubin and Albumin Levels in controls and in patients (pre and post-phototherapy) of neonatal jaundice

	Controls (Mean $\pm$ SD) Range	Before PT (Mean $\pm$ SD) Range	After PT (Mean $\pm$ SD) Range
Bilirubin (mg/dl)	1.34 ± 0.38 (0.8-2.0)	$15.43 \pm 2.21^*$ (12.3-20.0)	$9.42 \pm 1.2^{* **}$ (7.9-12.0)
Albumin (g/dl)	3.08 ± 0.27 (2.70-3.80)	3.06 ± 0.21 (2.60-3.40)	$2.92 \pm 0.16^{* \#}$ (2.50-3.30)
Bilirubin-Albumin Ratio ($\times 10^{-3}$)	0.44 ± 0.13 (0.24-0.71)	$5.07 \pm 0.89^*$ (3.73-7.31)	$3.23 \pm 0.5^{* **}$ (2.20-4.36)

* $p < 0.001$ as compared to controls, ** $p < 0.001$ as compared between two study groups,

$p < 0.01$ as compared between two study groups.

Table 2. Levels of MDA, Total Thiols, Reduced Glutathione, Vitamin C and SOD in controls and patients (pre and post-phototherapy) of neonatal jaundice

	Controls (Mean $\pm$ SD) Range	Before Phototherapy (Mean $\pm$ SD) Range	After Phototherapy (Mean $\pm$ SD) Range
MDA (nM/gHb)	3.37 $\pm$ 0.516 (2.59-4.60)	4.62 $\pm$ 0.58* (3.65-5.85)	5.63 $\pm$ 0.72* ** (4.08-6.85)
Total Thiols (M/gHb)	68.21 $\pm$ 7.38 (58.10-88.20)	47.46 $\pm$ 10.22* (36.88-65.20)	43.04 $\pm$ 6.27* ** (30.32-59.65)
Reduced Glutathione (M/gHb)	29.65 $\pm$ 4.88 (18.39-36.90)	14.83 $\pm$ 2.75* (11.61-21.12)	11.72 $\pm$ 2.86* ** (9.64-21.0)
Vitamin C (mg/gHb)	0.841 $\pm$ 0.16 (0.592-1.180)	0.445 $\pm$ 0.09* (0.296-0.640)	0.351 $\pm$ 0.07 * ** (0.22-0.51)
SOD (EU/gHb)	1377.90 $\pm$ 432.67 (1449-1625)	2212.57 $\pm$ 397.79* (1600-3020)	2580.27 $\pm$ 683.56* ** (1980-3890)

*p<0.001 as compared to controls, ** p<0.001 as compared between two study groups.

al. found no significant difference in the levels of serum MDA, vitamin E levels and other antioxidant enzymes (17, 18) in jaundiced neonates after phototherapy. This discrepancy in the results could be due to duration of phototherapy and feeding pattern.

Vitamin C levels showed a decrease from 0.445 $\pm$ 0.09 mg/gHb to 0.351 $\pm$ 0.07 mg/gHb after phototherapy (p<0.001). SOD levels in hemolysate showed a rise from 2212.57 $\pm$ 397.79 EU/gHb to 2580.27 $\pm$ 683.56 EU/gHb (p<0.001). The rise in the levels of SOD could be due to its induction to counter the effect of increased oxidative stress. Plasma albumin decreased from 3.06 $\pm$ 0.21g/dl to 2.92 $\pm$ 0.16 g/dl after phototherapy (p<0.01). Phototherapy, therefore, results in oxidative damage to red cells as indicated by significant decline in antioxidant status.

The results of the present study demonstrate that jaundice as well as phototherapy produces an increased oxidative stress which can be counteracted by increase in antioxidant response as evidenced by increase in SOD. Antioxidant defenses are highly stressed and less developed in infants (19); and phototherapy, by generating ROS, leads to their increased consumption.

Safe time period for phototherapy is not clearly defined in literature, however we followed the standard criteria followed in our setup (20). Preliminary clinical trials have shown that prevention of oxidative stress by using antioxidants failed to produce any encouraging effect and could not be considered as an effective therapy (19). Therefore, we recommend a very

cautious use of phototherapy in all patients of neonatal jaundice i.e. it should be administered only for the required duration and may be combined with antioxidant supplements. Large scale studies would be able to provide new insights in this field.

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