Phototherapy motivates protein and lipid oxidation in jaundiced term and late term neonates

Original Article

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Abstract:

Background: Hyperbilirubinemia is one of the most important complications encountered in neonatal units. It has been proposed that phototherapy yields oxidative stress. Therefore, this study was undertaken to survey the levels of antioxidant and oxidative stress in the serum of neonates before and after phototherapy.

Methods: This study was performed on thirty-five healthy, late preterm (>35 weeks) and term newborns aged 6-10 days, who underwent phototherapy due to hyperbilirubinemia (>14.00 mg/dL). Infants with a congenital malformation, birth asphyxia, sepsis, signs and symptoms suggestive of severe illness, and receiving phototherapy before recruitment to the study were excluded. Blood samples were taken to determine total serum bilirubin, total antioxidant capacity (TAC) of serum, malondialdehyde (MDA), advanced oxidation protein products (AOPP) as markers of the intensity of oxidative stress and inflammation with photometric methods, reduced and oxidized glutathione (GSH) by HPLC-UV as well as the ratio of them before and after phototherapy.

Results: TAC, GSH and bilirubin levels were significantly lower after phototherapy than before it, but reversely about levels of MDA, AOPP and oxidized GSH in addition to the ratio of reduced to oxidized GSH ($p<0.05$-0.001). AOPP and MDA showed a high negative correlation with bilirubin (respectively $R=-0.985$ and -0.986, $p<0.001$)) while vice versa about TAC and GSH ($R=0.975$ and 0.988, $P<0.001$).

Conclusion: Phototherapy induces oxidative stress and inflammation not only due to the elevation of protein and lipid oxidation but also with reducing of antioxidant markers of serum.

Key Words: Phototherapy, Oxidative stress, Hyperbilirubinemia, Malondialdehyde, Advanced oxidation protein products

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Introduction:

Bilirubin is a natural metabolic end-product of heme degradation. Bilirubin is an antioxidant at<6 mg/dL whereas it is responsible for oxidative stress and neurotoxicity at a higher level [1]. Neonatal jaundice is clinically observed in 84% of term newborns [2] and is the most prevalent reason for hospital readmission in the neonatal period. Severe hyperbilirubinemia (serum bilirubin level of more than 20 mg/dl) occurs in less than 2% of term infants and can lead to kernicterus and Permanent neurodevelopmental delay, as well as total serum bilirubin levels higher than 10 mg/dL, are presumed to be perilous for chromosomes [3, 4]. Therefore, evaluating all infants for hyperbilirubinemia is important [5]. Phototherapy is the most prevail treatment for lowering unconjugated bilirubin [6] although it may yield oxidative stress and mononuclear leukocyte DNA damage in jaundiced term

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infants [7-9]. Furthermore, antioxidant activity in the serum of neonates is lower than that of adults, and the neonatal erythrocyte membrane is more prone to oxidative damage thought its usual to pro-oxidant potential [10]. Free radicals and lipid and protein peroxides as products of them may be responsible for the pathogenesis of many conditions such as retinopathy of prematurity, bronchopulmonary dysplasia, intracranial hemorrhage, periventricular leukomalacia, sepsis, necrotizing enterocolitis, and hypoxic ischemic encephalopathy [11]. There are few and varying reports on changes of oxidative stress markers in serum of neonates with hyperbilirubinemia due to phototherapy [8, 10, 12]. Therefore, this study was undertaken to survey the levels of antioxidant and oxidative stress in serum of jaundiced neonate by measuring of malondialdehyde (MDA) and advanced oxidation protein products (AOPP) respectively as markers of lipid and protein oxidation, serum total antioxidant capacity (TAC) and reduced and oxidized glutathione (GSH) as well as ratio of them before and after phototherapy.

Materials:

All materials were in analytical grade and purchased from Sigma or Merck Chemical Companies.

Patients and samples

The present cross-sectional study was carried out in the neonatal unit of the Madani Hospital of Khorramabad, Iran during October 2015 to March 2016. According to Morgan table, the sample size was estimated 35 neonates (Confidence Level = 95%, Margin of Error = 5%). This study was performed on thirty-five healthy, late preterm (> 35 weeks) and term newborn infants aged 6-10 days, who underwent phototherapy due to hyperbilirubinemia (> 14 mg/dL). Infants with a congenital malformation, birth asphyxia, sepsis, those with signs and symptoms suggestive of severe illness, and babies who had received phototherapy before recruitment to the study were excluded. Approval of the local research ethics committee was obtained for the study and informed consent was signed by the parents. These babies achieved 48-hour continuous phototherapy (450 nm, 13 µW/cm2/nm; MicroLite Phototherapy System, Draeger). Phototherapy was interrupted only for feeding, cleaning and blood sampling.

Analytical methods

Two blood samples with 2 ml volume were taken from a peripheral vein to determine total serum bilirubin, reduced and oxidized glutathione (GSH), TAC, MDA and AOPP prior and 48-h after phototherapy. Blood samples were centrifuged at 1500 × g for 10 minutes within 20 minutes of collection. Serum samples were stored at -80°C until testing.

Total bilirubin was measured by the method of Doumas et al. [13], where bilirubin reacted with diazotized sulfanilic acid in the presence of caffeine with a final azo-pigment product. The developed color was read at 546 nm.

TAC was determined by the method of Benzie, et al. [14]. Briefly, a colorless ferric tripyridyltriazine complex is reduced to a blue ferrous complex by the antioxidants in the serum that modifying in absorbance at 593nm is directly correlated to the total reducing power in the sample.

Level of malondialdehyde (MDA) in serum was measured by the thiobarbituric acid (TBA) assay [15].

Determination of AOPP was based on the spectrophotometric detection, according to the method of Tylor, et al [16]. Serum samples were diluted to 10% in PBS and 300 µL applied in triplicate to a 96-well microplate. Standards of chloramine T (300 µL; 0–100 µM) were added to the plate and a sample blank was obtained by adding 300 µL PBS to the microplate. KI (1.16M, 15 µL) was added to all wells, followed 2min later by addition of 30 µL glacial acetic acid. The microplate was centrifuged (5800 g, 5min) to pellet precipitated protein, and 230 µL of the supernatant was transferred to a clean microplate. The absorption of the supernatant was read 10min at 340 nm after the addition of glacial acetic acid.

Oxidized and reduced glutathione (GSSG and GSH) were analyzed HPLC- UV [17].

Statistical analysis

All data were expressed as mean ± S.D. The statistical analysis of the data was executed with the Statistical Package for the Social Sciences, version 16.0, for Windows (SPSS, Inc). The Student's t-test for paired samples was used to compare the blood samples before and after phototherapy and Pearson rank correlation coefficient was used to compare the relationship between bilirubin with AOPP, MDA, GSH, and TAC using SPSS 16. $P < 0.05$ was considered significant.

Results:

This study comprised 35 infants (18 males and 17 females), aged 6-10 days. Serum antioxidant/oxidant parameters and bilirubin before and after phototherapy are presented in Table .1. Bilirubin, TAC, and reduced GSH levels were significantly lower after phototherapy than before it. Reversely, MDA, AOPP and oxidized GSH concentrations were higher after phototherapy than before it. The ratio of reduced GSH to oxidized

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GSH or GSH/GSSG is shown in Figure 1. The ratio of GSH/GSSG notable reduced after phototherapy in the jaundiced neonates (p<0.05-0.001). Figure 2 presented the correlation between bilirubin and other cited parameters. In this study, MDA and AOPP showed a high negative correlation with bilirubin (respectively, R=-0.985 and -0.986, p < 0.005) vice versa about TAC and GSH (R=0.975, 0.988 P<0.001).

Table.1. Oxidative stress markers (MDA and AOPP) and Total antioxidant capacity of serum in the jaundiced neonates before and after phototherapy

Parameters	Photography		P value<A vs B
	Before (B)	After (A)	
Bilirubin (mg/dL)	14.41±1.09	8.73±0.60	0.001
MDA (µmol/L)	2.80±0.18	3.76±0.23	0.05
AOPP (µmol/L)	206.54±11.76	290.13±15.85	0.001
GSH (mmol/L)	1.94±0.12	1.27±0.07	0.05
GSSG (µmol/L)	0.52±0.11	0.93±0.19	0.001
TAC (µmol/L)	1.12±0.07	0.77±0.04	0.001

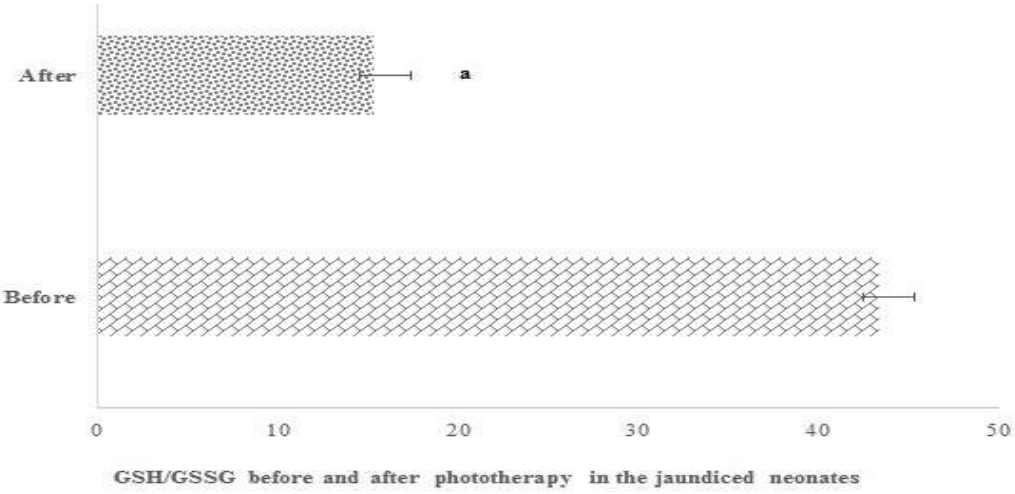

Fig 1. Ratio of reduced glutathione to oxidized of it (GSH/GSSG) in the jaundiced neonates before and after phototherapy

a Indicates significance of data comparing before with after phototherapy

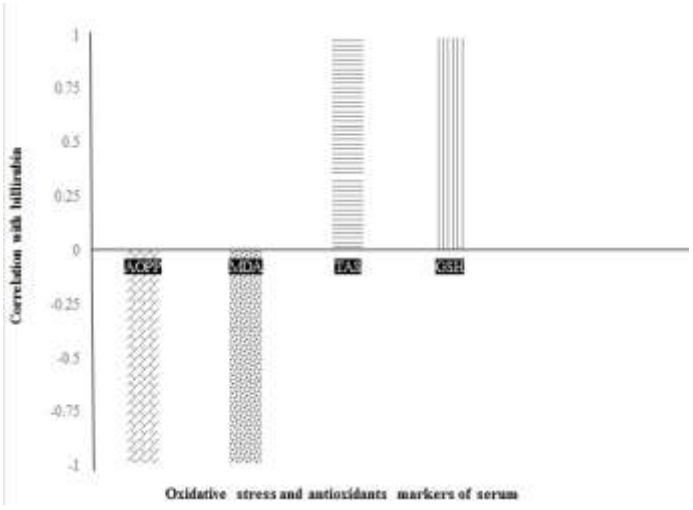

Fig.2. Correlation of advanced oxidation protein products (AOPP), malondialdehyde (MDA), the total antioxidant capacity of serum (TAC) and glutathione (GSH) with total bilirubin.

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Discussion:

In this study, phototherapy declined serum bilirubin along with multiplying AOPP and MDA in addition to the diminishing of the total antioxidant capacity of serum and the ratio of reduced GSH to its oxidized form (GSSG) (Table.1 & Fig.1). Further, it has not only subsided serum antioxidant level but also induced cellular injury following to protein and lipid oxidation ($p<0.05$-0.001). The effect of phototherapy on oxidized GSH in addition to the ratio of reduced GSH of its oxidized form, for the first time, has been reported by us as well as the effect on AOPP has been only reported in a survey performed before the present study [18].

We selected ratio of GSH to GSSG and TAC as markers of antioxidants status of serum as well as AOPP and MDA as markers of oxidative stress for better investigation of the oxidative effect of phototherapy on the hyperbilirubinemia neonates. On the other hand, the levels of these markers exhibited the activity of the antioxidant enzyme. Based on our results, alternations of all antioxidative and oxidative stress markers without contradiction presented the oxidative effect of phototherapy on jaundiced neonates. Furthermore, there are some studies with controversial results between cited parameters [8, 10, 12]. In accordance with our results, it was reported previously that phototherapy persuaded oxidative stress with the raising of MDA and AOPP as well as decreasing of GSH and superoxide dismutase (SOD) in erythrocytes and serum of neonatal jaundice [8, 18-20]. Further, phototherapy in cultured cells induced the generation of more photo-oxidation products and caused more severe cellular damage in the presence of bilirubin [21]. AOPP as markers of oxidative damages to proteins, the intensity of oxidative stress and inflammation [22]. Furthermore, phototherapy prompted oxidative stress concomitant with inflammation that previous studies only reported its oxidative effect [8, 18, 19].

Based on Figure 2, a high negative correlation can be observed between oxidative stress markers (AOPP and MDA) and total bilirubin, but reverse about TAC and GSH ($P<0.001$). A similar result has been shown about the relation between bilirubin with MDA [23] and TAC. The correlation between bilirubin with AOPP and GSH has been reported to us for the first time. A notable positive correlation between bilirubin with TAS and GSH whereas inversely about MDA and AOPP, displaying that the bilirubin has a significant influence on the TAC and oxidative stress. However, no correlation was observed between total antioxidant capacity and serum total bilirubin in babies with a value greater than 20 mg/dL [12]. Therefore, hyperbilirubinemia may augment antioxidant defense in vivo [24]. Phototherapy as widespread treatment for declining no-direct bilirubin in jaundiced newborns induces cell injury not only with lipid and protein oxidation but also by generation of inflammatory events. It is suggested that pretreatment with antioxidants may reduce induction of oxidative stress due to phototherapy.

The present data exhibit that phototherapy induces oxidative stress and inflammation not only due to the elevation of MDA and AOPP but also by reducing of TAS and the ratio of reduced GSH to its oxidized form. It is suggested that supplementation of antioxidants before phototherapy may reduce oxidative stress. A high positive correlation of GSH and TAS with total bilirubin, but reversely AOPP and MDA presented that bilirubin level has an important effect on the TAC and oxidative stress.

The limitations of the study:

The limitations of the study were that a) the study was managed only on preterm and term infants who needed phototherapy without a control group b) antioxidative and oxidative markers were just determined 48 hours after phototherapy.

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