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Original Article

Oxidative stress in neonates with hyperbilirubinemia before and after phototherapy: malondialdehyde and catalase activity

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Abstract

Background Phototherapy is used to treat neonatal hyperbilirubinemia, but is currently thought to cause photodynamic stress and can induce lipid peroxidation. There is increasing evidence that many severe diseases of the neonates are caused by oxidative injury and lipid peroxidation. In the present communique, we review the oxidative succeptibility of the neonates and the evidence now available that phototherapy induces oxidative stress. Malondialdehyde (MDA) is a metabolic product of free radicals. Catalase is an antioxidant that binds free radicals.

Objective To compare the levels of oxidants and antioxidants before and after phototherapy in neonates with hyperbilirubinemia.

Methods This pretest-posttest control group study was conducted in Sanglah Hospital, Bali, from November 2016 to April 2017. Thirty babies with gestational age ≥35 weeks and hyperbilirubinemia with total bilirubin levels requiring phototherapy were included in this study. The MDA levels and catalase activity were measured before and after 24 hours of phototherapy.

Results Comparative analysis using paired T-test showed a significant increase of malondialdehyde level, with mean MDA 23.73 (SD 8.20) nmol/mL before and 53.05 (SD 10.18) nmol/mL after phototherapy (P<0.001). However, catalase activity significantly decreased from of 72.33 (SD 10.63) kU/L before phototherapy to 44.85 (SD 14.79) kU/L after phototherapy (P<0.001). The MDA level had a significant, negative association with catalase activity after phototherapy (r =-0.4; P=0.028).

Conclusion Neonates with hyperbilirubinemia are found to have increased oxidative stress after phototherapy, as indicated by increased MDA levels and decreased CAT activity after 24 hours of phototherapy. **[Paediatr Indones. 2018;58:269-73; doi: http://dx.doi.org/10.14238/pi58.6.2018.269-73]**.

Keywords: *hyperbilirubinemia; oxidative stress; malondialdehyde; catalase activity; phototherapy*

Phototherapy is the treatment of choice for hyperbilirubinemia in neonates. It is noninvasive, easy to perform, has few side effects, and is low cost.[1] Some studies have suggested that phototherapy can lead to increased oxidative stress and lipid peroxidation.[2]

Oxidative stress occurs as a result of an imbalance between oxidants, antioxidants, and free radical production. Neonatal red blood cell membranes are more likely to suffer from oxidative stress because it is the dominant pro-oxidants. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro-oxidant potential. The antioxidant activity in serum in term infants is lower compared to that of adults.[3] Oxidative stress can affect lipids, proteins, and DNA, and is believed to play a role in the occurrence of various diseases.[3]

Malondialdehyde (MDA) is a frequently-used marker of oxidative stress and lipid peroxidation in

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vivo.[4] Catalase belongs to the class of hydroperoxidase enzymes that can catalyze the breakdown of hydrogen peroxide (H_2O_2) substrate to water and oxygen, thereby preventing lipid peroxidation of the cell membrane and acting as a free radical binder.[5] The effect of phototherapy on lipid peroxidation remains unclear. One study found a significant increase in MDA levels and parameters of oxidants and a decrease in antioxidant levels after phototherapy.[3] Another study found decreased levels MDA after phototherapy, but increased levels of nitric oxide (NO).[6] Therefore, we aimed to compare malondialdehyde levels and catalase activity in infants with hyperbilirubinemia, before and after phototherapy.

Methods

This pretest-posttest control group design study was conducted from November 2016 to April 2017 in the Neonatal Ward, Sanglah Hospital, Bali. It was approved by the Ethics Review Board at Udayana University, Bali. Subjects were neonates with hyperbilirubinemia requiring phototherapy selected by consecutive sampling. Sample size was calculated based on the two-paired group study formula with type one error of 5% ($Z\alpha=1.96$), type 2 error of 10% ($Z\beta=1.28$), standard deviation of 0.72 and minimum mean differences of 0.45. The minimum required number of subjects was 30 children.

Neonates included in the study had gestational age ≥ 35 weeks, birth weight ≥ 2,500 grams, hyperbilirubinemia requiring phototherapy, and jaundice within days 2-14 of life. Exclusion criteria were neonates with total serum bilirubin (TSB) levels exceeding phototherapy levels and necessitating exchange transfusion, severe asphyxia, major congenital anomalies, or sepsis.

All subjects underwent full history-taking (including medical problems during pregnancy, mode of delivery, delivery events and resuscitation, family history, and postnatal age) and detailed clinical examinations (including birth weight, general examination, as well as chest, heart, abdominal and neurological examinations). Serum bilirubin, MDA, and catalase activity levels were measured at the time of admission (before exposure to phototherapy) and 24 hours after exposure to continuous phototherapy, with the assistance of the clinical pathology laboratory.

Malondialdehyde was measured by a spectrophotometric assay, using the principle that lipid products react with thiobarbituric acid to give a red chromogen, with absorbance at 532 nm. Catalase activity was estimated using an ELISA assay.[2,4,7] Phototherapy was performed for 24 hours, using fluorescent lamps 4 x 20 W as blue light sources, with a wavelength of 420-470 um and light intensity of 10 μW/cm2/nm. The lamp was placed at a distance of 20 cm from the baby's body.

Data was processed with *SPSS 20.0*. Descriptive data were presented in text and tables. Paired student T-test was used to compare bilirubin, MDA, and catalase activity before and after phototherapy. Pearson's correlation test was used to analyze the strength of correlation.

Results

During the study period we enrolled 30 neonates who met the inclusion criteria. Characteristics of subjects are shown in **Table 1**. Two (6.7%) subjects experienced a skin rash and one (3.3%) had hyperthermia during phototherapy. The mean bilirubin level before phototherapy was 18.77 (SD 0.61) mg/dL and after phototherapy was 10.56 (SD 0.72) mg/dL ($P<0.05$). Mean MDA level significantly increased from before [23.73 (SD 8.20) nmol/mL] compared to after phototherapy [53.05 (SD 10.18) nmol/mL] ($P<0.001$). In addition, catalase activity significantly decreased from before [72.33 (SD 10.63) kU/mL] compared to after phototherapy [44.85 (SD 14.79) kU/mL] ($P<0.05$) (**Table 2**). Pearson's correlation

Table 1. Characteristics of subjects

Characteristics	(N=30)
Sex, n	
Male	16
Female	14
Mean birth weight (SD), grams	2955 (325)
Mean age at appearance of jaundice (SD), days	3 (0.5)
Types of delivery, n	
Vaginal	19
Caesarean section	9
Vacuum extraction	2

Table 2. The mean levels of bilirubin, MDA, and catalase activity, before and after phototherapy

Variables	Before phototherapy	After phototherapy	P value*
Mean bilirubin (SD), mg/dL	18.77 (0.61)	10.56 (0.72)	<0.001
Mean MDA (SD), nmol/mL	23.73 (8.20)	53.05 (10.18)	<0.001
Mean CAT activity (SD), kU/L	72.33 (10.63)	44.85 (14.79)	<0.001

*= between before and after phototherapy group (paired T-test)

test revealed a negative correlation between increased MDA levels and catalase activity levels (r=-0.4; P=0.028).

Discussion

We found a significant increase in mean MDA level from before [23.73 (SD 7.33) mmol/mL] to after phototherapy [53.05 (SD 10.18) mmol/mL]. Thiagarajan *et al.* (2014) also noted a significant increase in mean plasma MDA levels after 48 hours of phototherapy [before 12.61 (SD 2.32) μmol/L and after 13.79 (SD 2.85) mmol/L].7,8 In addition, Dahiya et al. reported a significant increase in mean MDA level after 48-96 hours of phototherapy [before 4.62 (SD 0.52) nM/gHb vs. after 5.63 (SD 0.72) nM/gHb] (P<0.001).[1,2,4]

Increased levels of MDA occur due to the formation of free radicals during phototherapy. These free radicals bind to unsaturated fatty acids in the red blood cell membrane, causing lipid peroxidation. Production of free radicals due to phototherapy coupled with low antioxidant defenses in the neonate causes oxidative stress. The reactive oxygen species (ROS) react with lipids, proteins and DNA, producing lipid radicals, alkali-based radicals and sugars, fatty acid radicals, and other radical types. These radical products produce peroxyl radicals when reacting with oxygen, which contribute to oxidative damage. When ROS react with the lipid membrane, the lipid peroxidation results in the formation of lipid hydroperoxide (LOOH), which decomposes into aldehyde forms such as malondialdehyde, 4-hydroxy nonenal (4-HNE) or cyclic endoperoxide, isoprotan, and hydrocarbon forms. Malondialdehyde is the end product of lipid peroxidation processes that can be used as a marker for describing oxidative stress conditions.[2,3]

Abdel Latief *et al.* observed a significant decrease in MDA levels after phototherapy for 12 hours, with 3.28 (SD 0.62) nmol/L before and 2.54 (SD 0.51) nmol/L after phototherapy (P<0.001). This difference to our results may have been due to different phototherapy durations, 12 hours of phototherapy in their study and 24 hours in ours.[6,7,9] The decrease in MDA levels was likely due to improvements in the condition of hyperbilirubinemia and new oxidative stress occurring after greater administration of phototherapy.[8,10]

Several studies have compared oxidative and antioxidant test results before and after phototherapy. We observed a significant decrease of mean CAT activity, with 72.33 (SD 10.63) kU/L before and 44.85 (SD 14.79) kU/L after phototherapy. Dahiya *et al.* also noted that, in addition to increased MDA levels, antioxidant components were significantly decreased after received phototherapy ranging from 48 to 96 hours, such as reduced glutathione (GSH), total thiols, and vitamin C. Supporting results were also obtained by Gulbayzar *et al.* and Block *et al.* Aycicek *et al.*, with significantly decreased antioxidant components such as vitamin C, uric acid, and total antioxidant capacity (TAC), as well as significantly increased total oxidant status (TOS), lipid hydroperoxide, and oxidative stress index after phototherapy for 48 hours (P<0.05).[4,5]

The antioxidant system is classified into two major groups: enzymatic and non-enzymatic antioxidants. A small portion of the oxygen consumed for the aerobic process is converted to an anionic superoxide that must be converted to a less reactive molecule. The major enzymes regulating this process are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase. The SOD is considered to be first line defense against ROS. This enzyme is present in almost all cells, and converts O_2- into H_2O_2. Mitochondria and SOD bacteria contain Mn, while SOD cytosol is a dimer containing Cu and Zn. The SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre. As H_2O_2 may react with other ROS, it needs to be degraded by one of the other two antioxidant enzymes, GSH-Px or catalase. GSH-Px is located in the mitochondria and

catalyzes the degradation of H_2O_2 by reduction, in which two gluthathione (GSH) molecules are oxidized into glutathione disulfide (GSSG).[7-9]

Pearson's correlation test revealed a significant negative relationship between MDA and CAT activity levels, before and after phototherapy (r=-0.4; P=0.028). Dahiya *et al.* and Thiagarajan *et al.* had similar results in their studies.[3,4,6] Under normal conditions, free radicals produced in the body are neutralized by antioxidants. When free radical levels increase due to phototherapy, the ability of endogenous antioxidants is inadequate to neutralize free radicals, resulting in an unbalanced state between free radicals and antioxidants, which is called oxidative stress. Since MDA is an end product of lipid peroxidation, increased MDA levels and decreased CAT activity are indicative of oxidative stress. Despite the lack of clinical symptoms in our subjects, the damage to lipids, proteins, and DNA occurs at the molecular level. Mutations and damage to DNA may lead to clinical manifestations later in life, especially in those with repeated exposure to conditions causing increased oxidative stress. Such oxidative reactions may also have a role in the occurrence of various allergic diseases (asthma, allergic rhinitis, or conjunctivitis) during childhood.[1,3,4]

A limitation of this study was that while we planned for phototherapy to be done for a full 24 hours, in reality, the implementation of phototherapy was <24 hours, because phototherapy was stopped while mothers breastfed their infants. We did not record the frequency and duration of breastfeeding. One of the management recommendations for hyperbilirubinemia is hydration with breastfeeding on demand. The volume of breast milk given was also not calculated, as it is impossible to do so, hence, study subjects likely received non-standardized volumes of milk.[7,10]

In conclusion, neonates with hyperbilirubinemia are found to have increased oxidative stress after phototherapy, as indicated by increased MDA levels and decreased CAT activity after 24 hours of phototherapy. Therefore, we recommend a very cautious use of phototherapy in all patients with neonatal jaundice.

Conflict of Interest

None declared.

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