# Conventional Versus Intensive Phototherapy and Oxidative-stress Status in Neonates with Unconjugated Hyperbilirubinemia

*Thesis*

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| *APP* | *American Academy Of Pediatrics* |
| *Bpm* | *Beat per minute* |
| *Ca* | *Calcium* |
| *CBC* | *Complete blood count* |
| *Cm* | *Centimeter* |
| *CNS* | *Central nervous system* |
| *C°* | *Celsius* |
| *CRT* | *Capillary refill time* |
| *CSF* | *Cerebrospinal fluid* |
| *Cu* | *Copper* |
| *D.Bil* | *Direct bilirubin* |
| *DAT* | *Direct antiglobulin test* |
| *DBP* | *Diastolic blood pressure* |
| *Fe* | *Iron* |
| *G6PD* | *Glucose-6-Phosphate Dehydrogenase* |
| *GS* | *Glutamine synthetase* |
| *GTH* | *Glutathione* |
| *Hb* | *Hemoglobin* |
| *Hct* | *Hematocrit* |
| *HE* | *Hereditary elliptocytosis* |
| *HR* | *Heart rate* |
| *ID* | *Iron deficiency* |
| *IVIG* | *Intravenous immunoglobulin* |
| *Kg* | *Kilogram* |
| *LED* | *Light-emitting diode* |
| *MDA* | *Malondialdehyde* |
| *Mmhg* | *Millimeter mercury* |
| *NA* | *Not applicable* |
| *NAD* | *No abnormality detected* |
| *NO* | *Nitric Oxide* |
| *PDA* | *Patent ductus arteriosus* |
| *Plt* | *Platelets* |
| *RBC* | *Red blood cell* |

# List of Abbreviations cont…

| Abb. | Full term |
|---|---|
| *RCT* | *Randomized controlled trial* |
| *RNS* | *Reactive nitrogen species* |
| *ROS* | *Reactive oxygen species* |
| *RR* | *Respiratory rate* |
| *SBP* | *Systolic blood pressure* |
| *Sec* | *Second* |
| *SOD* | *Superoxide Dismutase Enzyme* |
| *TAC* | *Total Antioxidant Capacity* |
| *TBA* | *Thiobarbituric acid* |
| *TBARS* | *Thiobarbituric Acid Reactive Substances* |
| *TcB* | *Transcutaneous bilirubin* |
| *Temp* | *Temperature.* |
| *TLC* | *Total leucocytic count.* |
| *TSB* | *Total serum bilirubin* |
| *UCB* | *Uncomjugated bilirubin* |
| *UDPG* | *Uridine diphosphate glucuronyl transferase* |
| *UGT1A* | *Uridine diphosphoglucuronosyl transferase 1A1* |
| *Zn* | *Zinc* |

## Abstract

**Background:** Neonates have limited antioxidant protective capacity. It has been demonstrated that phototherapy used for treatment of neonatal jaundice produces oxidative stress. Various phototherapy devices using different light sources are available for phototherapy. **Aim:** To investigate the effect of different light sources on the lipid peroxidation and antioxidant enzyme activities in neonates with unconjugated hyperbilirubinemia before and 48 hours after phototherapy. **Methods:** 80 Term and late-preterm (≥35 weeks) newborn infants with unconjugated hyperbilirubinemia hospitalized to receive phototherapy according to American Academy of Pediatrics guidelines were enrolled. Infants were randomly assigned to 2 groups: 40 neonates received conventional phototherapy and the other 40 neonates received intensive phototherapy (Bilisphere 360). The total serum bilirubin (TSB), direct serum bilirubin (DSB), total antioxidant capacity (TAC), malonaldehyde (MDA), nitric oxide (NO), iron (Fe), copper (Cu), zinc (Zn) and calcium (Ca) were measured before and 24 h after phototherapy. **Results:** TAC declined significantly in both groups post-phototherapy ($p<0.05$) with no statistical difference between both groups ($p=0.159$). Moreover, MDA and NO increased significantly post-phototherapy in both groups more in intensive phototherapy group ($p<0.05$). Furthermore, correlation analysis showed positive correlation between TSB and TAC ($p<0.05$). On the other hand, there was negative correlation between TSB and both of MDA and NO ($p<0.05$). **Conclusion:** As indicated by declined TAC and increased MDA and NO, oxidant/antioxidant balance is disturbed in favor of oxidant after conventional and intensive phototherapy. That increased oxidative stress in neonates leads to decrease in the levels of antioxidants, that weaken their ability to fight the growing stress.

# INTRODUCTION

Jaundice is a common and mostly benign condition in neonates that occurs in more than 60 % of all neonates. It is characterized by yellowish discoloration of the skin and conjunctiva as a consequence of increased levels of serum bilirubin during the first week of life ***(Greco et al., 2016)***.

Most of these babies have 'physiological jaundice', which typically becomes clinically apparent on day 3, peaks on day 5 to 7, resolves by day 14 and is usually benign ***(AAP, 2004).***

Bilirubin has an antioxidant activity that is anticipated by cycling between bilirubin and biliverdin. Bilirubin interacts with reactive oxygen species (ROS), neutralizing its toxicity and transforms to biliverdin, which is reduced by biliverdin reductase enzyme to regenerate bilirubin ***(Nag et al., 2009).***

Phototherapy has been used since 1958 for the treatment of neonatal hyperbilirubinaemia ***(Cremer et al., 1958)***. Its noninvasive nature, easy availability, low cost and occurrence of few side effects have initially almost led to the assumption that it is innocuous ***(Tan, 1991)***. It causes unconjugated bilirubin to be mobilized from the skin by structural isomerization to a water soluble form that can be excreted in the urine. Lamps emitting light between the wavelengths of 400 - 500 nanometers (peak at 460nm) are specifically used for

administering phototherapy as bilirubin absorbs this wavelength of light ***(Verman et al., 2004).***

Phototherapy might have a negative impact on the oxidant/antioxidant defense system in hyperbilirubinemic infants with the resultant exposure to potent oxidative stress ***(Aycicek and Erel, 2007)***, where there is an imbalance between concentrations of reactive oxygen species (ROS)/reactive nitrogen species (RNS) and antioxidants. This excessive ROS/RNS accumulation will lead to cellular injury, such as damage to deoxyribonucleic acid, proteins, and lipid membranes ***(Trachootham et al., 2008)***. Elevated levels of RNS have been implicated in cell injury and death by inducing nitrosative stress. One of the most important RNS is nitric oxide (NO) ***(Martínez and Andriantsitohaina, 2009).***

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues ***(Draper and Hadley, 1990).*** It can be described generally as a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond, especially polyunsaturated fatty acids ***(Lu et al., 2010).*** Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is malondialdehyde (MDA) which is the product of the

breakdown of phospholipids that is preferred as a marker of oxidative stress ***(Draper and Hadley, 1990)***.

Antioxidant defenses keep a check on the generation of ROS. An antioxidant is a substance that is present at low concentrations and significantly delays or prevents oxidation of the oxidizable substrate ***(Kohen and Nyska, 2002).***

Total antioxidant capacity (TAC) summarizes overall activity of antioxidants and antioxidant enzymes. The depletion of TAC induced by oxidative stress is eliminated by release of stock organ antioxidants, mainly from liver and adipose tissue and the induction or activation of antioxidant enzymes. At a later phase of oxidative stress, the TAC falls due to depletion of antioxidants. Therefore, determination of TAC provides information about antioxidant types and their concentration without exact qualitative differentiation ***(Zima et al., 1996; Cao and Prior, 1998).***

Essential trace elements such as copper, zinc, iron, manganese, are antioxidant micronutrients that are crucial for growth, carbohydrate and protein metabolism, gene transcription, endocrine function and nutrient transport in humans ***(Keen et al., 2003).***

# Aim of the Work

The aim of the present study is to test a hypothesis assuming that phototherapy might have an effect on oxidant/antioxidant status in term and late-preterm neonates with unconjugated hyperbilirubinemia.

Chapter One

# NEONATAL UNCONJUGATED HYPERBILIRUBINEMIA

Jaundice usually becomes visible on the sclera at a level of 2 to 3 mg/dL (34 to 51 μmol/L) and on the face at about 4 to 5 mg/dL (68 to 86 μmol/L). With increasing bilirubin levels, jaundice seems to advance in a head-to-foot direction, appearing at the umbilicus at about 15 mg/dL (258 μmol/L) and at the feet at about 20 mg/dL (340 μmol/L), it is also observed during 1st wk of life in approximately 60% of term infants and 80% of preterm infants ***(Piazza and Stoll, 2008).***

## Metabolic pathway of bilirubin formation:

### *I. Source of bilirubin:*

- Bilirubin is derived from breakdown of heme-containing proteins in the reticuloendothelial system (RES). The normal newborn produces 6 to 10 mg of bilirubin per kilogram per day as opposed to production of 3 to 4mg/kg/day in adult.
- The major heme-containing protein in red blood cell (RBC) is hemoglobin (Hb). Hb released from senescent RBCs in the RES. is the source of 75% of all bilirubin production. One gram of Hb produces 34 mg of bilirubin. Accelerated release of Hb from RBCs is the cause of