Effect of Phototherapy on CD4, CD8 and Natural Killer Cells of Full Term Neonates with Indirect Hyperbilirubinemia

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Phototherapy is generally considered a very safe and well-tolerated treatment for hyperbilirubinaemia. However, clinical users should be aware of the unwanted effects of using phototherapy. Affection of neonatal immune system due to phototherapy has been reported. This study aimed to evaluate the effect of phototherapy on level of CD4⁺, CD8⁺ and natural killer (NK) (CD16⁺ & CD65⁺) lymphocytes subsets in neonates. The number of these lymphocytes was measured 72 hrs after phototherapy exposure in 30 full term neonates with indirect hyperbilirubinemia and compared to those of 25 healthy controls using flow cytometry. Results showed non-significant changes of the tested lymphocyte subsets after 72 hrs exposure to phototherapy. In conclusion, phototherapy has no significant effect on the level of circulating CD4⁺, CD8⁺ and NK lymphocytes.

Jaundice is the most common condition that requires medical attention in newborns. The yellow coloration of the skin and sclera in newborns with jaundice is the result of accumulation of unconjugated bilirubin. In most infants, unconjugated hyperbilirubinemia reflects a normal transitional phenomenon. However, in some infants, serum bilirubin levels may excessively rise, which can be cause for concern because unconjugated bilirubin is neurotoxic and can cause death in newborns and lifelong neurologic sequelae in infants who survive (Porter et al., 2002).

Phototherapy is the primary treatment in neonates with unconjugated hyperbilirubinemia. This therapeutic principle was discovered in England in the 1950s and is the most widespread therapy used in newborns (Mreihil et al., 2010). Phototherapy is generally safe, but some investigators have expressed concern about its potential toxic effect (Cetinkursun et al., 2006). One possible harmful consequence is affection of cytokines production and lymphocytes subtypes which can influence the function of the immune system in newborns (Kurt et al., 2009). Phototherapy increases DNA damage in lymphocytes of hyperbilirubinemic neonates (Tatli et al., 2008). A possible genotoxic effect for phototherapy has been demonstrated in experimental (Christensen, 1994) and observational studies (Tatli et al., 2008).

Peripheral blood lymphocytes are commonly used to monitor environmentally induced genetic damage (Cole, 1994).

The aim of this study was to evaluate the effect of phototherapy on level of CD4⁺, CD8⁺ and natural killer (NK) (CD16⁺/CD65⁺) lymphocytes subsets in neonates.

Materials and Methods

This prospective case-control study was conducted on 55 full term newborns obtained from Aga District Hospital, Dakhlia governorate, Egypt over a period of eight months from August 2014 to March 2015. They were classified into two groups:

Neonates exposed to phototherapy Group: thirty full term newborns with neonatal indirect hyperbilirubinemia.

Neonates not exposed to phototherapy Group: twenty five full term healthy newborns.
Control Group: twenty five healthy full term newborns without neonatal jaundice.

Informed consent was obtained from parents of every neonate included in this study. Approval from Ethical Committee of Menoufia Faculty of Medicine was obtained.

Inclusion criteria: Full term neonates: 37-41 weeks and 6/7 days (260-294 days), Appropriate for gestational age: 10th to 90th percentile and indirect Hyperbilirubinemia in need of phototherapy according to guidelines obtained from American Academy of Pediatrics subcommittee on Hyperbilirubinemia (AAP, 2004).

Exclusion criteria: Preterm neonates: less than 37 weeks completed weeks (259 days), Post term neonates: 42 weeks (295 days) or more, Small for gestational age and large for gestational age, Direct hyperbilirubinemia, Neonates subjected to exchange transfusion, Neonates suffering from any systemic illness other than neonatal jaundice, i.e. infant of diabetic mother, congenital pneumonia, congenital infections and birth asphyxia, Neonates suffering from any serious congenital anomalies and sepsis.

Both groups were subjected to full history taking, clinical examination & laboratory investigations (Total and direct serum Bilirubin, Blood group, Rh antigen, Coomb’s test and immunophenotypic analysis of CD4, CD8, CD16 & CD56 by flow cytometry).

Blood samples were collected from neonates exposed to phototherapy before and after 72 hrs of exposure to phototherapy (the longest period for most of cases treated by phototherapy) and from controls at time of examination.

Phototherapy

Phototherapy was used based on the AAP guidelines (AAP, 2004). The phototherapy system (Phototherapy Unit; 520 CobamsSrI, Bologna, Italy) consisted of six fluorescent special blue light lamps (Philips F20T12/BB). The phototherapy intensity (=10 μW/cm2/nm) and spectrum were centered around 450–560 nm. None of our infants received LED phototherapy. The phototherapy system was placed over the infants, at a distance of 40 cm. The infants were placed naked under phototherapy, with their eyes and genitals covered. All patients were exposed to continuous phototherapy, except during the time of feeding, cleaning, and blood sampling. Phototherapy was stopped when TSB levels were below 2 mg/dL from the lowest limit for phototherapy.

Enumeration of circulating CD4, CD8, CD16 and CD56 positive cells

Detection of CD4, CD8, CD16 and CD56 was performed using monoclonal antibodies (Dako, Denmark). This test depends on the ability of a monoclonal antibody to bind to the surface of the cells expressing CD4, CD8, CD16 and CD56 lymphocytes and measured by flow cytometry using the Becton-Dickinson Fluorescence activated cell sorter (FACS Calibur, BD immune cytometry systems, USA).

- Monoclonal antibodies
  *FITC (fluorescein isothiocyanate) conjugated mouse monoclonal anti-human CD4, code F0766, clone MT310 for flowcytometry, DAKO, Denmark.
  *PE (phycoerythrin) conjugated mouse monoclonal Anti-Human CD8 PE, code R0806 Clone DK25 for flowcytometry, DAKO, Denmark.
  *FITC conjugated mouse monoclonal Anti-Human CD16, codeF7011, clone DJ130c for flowcytometry, DAKO, Denmark.
  *PE conjugated mouse monoclonal Anti-Human CD56, code R7251 Clone C5.9 for flowcytometry, DAKO, Denmark.

- Sample preparation

Two ml of EDTA blood was layered carefully on top of 1 ml of Ficoll (type 400, 20% in H2O Sigma-Aldrich) and then centrifuged for 20 min at 500 g. Separation of mononuclear layer was carried out in a separate tube, washed twice with 3 ml of PBS and centrifuged for 5 min at 1200 g. The supernatant was decanted and the sediment was suspended in 500ul of PBS.

- Sample staining

For each sample three tubes were prepared: First one for simultaneous detection of CD4 and CD8 expression using FITC-labeled anti CD4 Ab and PE-labeled anti CD8 Ab. The second tube was used for simultaneous detection of CD16 and CD56 expression using FITC-labeled anti CD16 Ab and PE-labeled anti CD56 Ab. In the third tube, isotypic control was used to exclude the cell autofluorescence, instrument noise and non specific binding.

A volume of 10 μl of monoclonal Ab was added to 100 μl of the previously prepared cell suspension, mixed well, and incubated at 2-8°C for 30 min in the dark; the cells were then washed three times in 2 ml of PBS.

Finally the cells were suspended in 200 μl of PBS for final flowcytometric analysis. All samples were analyzed using a flowcytometer (Becton Dickinson FACS Calibur, USA) in Hematology and Oncology...
Unit, Pediatric Department, Faculty of Medicine, Menoufia University.

- Flowcytometric analysis

Data were acquired on a FACS caliber flow cytometer (BD immune cytometry systems, San Jose, CA). The instrument set up was checked weekly using QC windows beads (flowcytometry standard, San Juan, PR). Forward scatter and side scatter measurements were made using linear amplifiers, and fluorescence measurements were made with logarithmic amplifiers.

Gating was done on the lymphocytes. Two colors and light scattering properties were applied to determine CD4\(^+\) T cells, CD8\(^+\) T cells and CD16\(^+\)/CD56\(^+\) NK cells. Data were acquired from 10000 cells. Results were presented as the average percentage of total lymphocytes. Absolute number of each lymphocyte subset was calculated as the product of its percentage multiplied by total number of lymphocytes in blood, (figure 1).

**Statistical Analysis**

The program used was SPSS version 16. Quantitative data were analyzed using mean and standard deviation. Student t test was used to compare means of different groups, a value of \( P < 0.05 \) were considered to indicate statistical.

**Results**

There was no significant difference in CD4\(^+\), CD8\(^+\) and CD16\(^+\)/CD56\(^+\) levels between neonates exposed to phototherapy and controls. Within the neonates exposed to phototherapy group, no significant difference in CD4\(^+\), CD8\(^+\) and CD16\(^+\)/CD56\(^+\) levels was found between the before and after phototherapy levels, (Table 1).

Figure 2 shows statistically non-significant difference in CD4\(^+\) cells (X axis, lower right quadrant) between a case before phototherapy (a), after phototherapy (b) and a control (c).

Also, statistically non-significant difference in CD8\(^+\) cells (Y axis, upper left quadrant) between neonates before phototherapy (a), after phototherapy (b) and a control (c).

Figure 3 shows statistically non-significant difference in CD16\(^+\)/56\(^+\) cells (upper right quadrant) between neonate before phototherapy (a), after phototherapy (b) and a control (c).
Table 1. CD4+, CD8+ and CD16+/CD56+ levels in neonates exposed to phototherapy and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neonates exposure to phototherapy</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Range</td>
<td>$\bar{x} \pm SD$</td>
</tr>
<tr>
<td></td>
<td>before</td>
<td></td>
</tr>
<tr>
<td>CD4+ (cells/mm$^3$)</td>
<td>1.131-2.293</td>
<td>1.712± 451</td>
</tr>
<tr>
<td>CD8+ (cells/mm$^3$)</td>
<td>611-1101</td>
<td>856± 241</td>
</tr>
<tr>
<td>CD16+/CD56+ (cells/mm$^3$)</td>
<td>483-907</td>
<td>695± 218</td>
</tr>
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CD4+: Normal range (500-2400), CD8+: Normal range (200 to 1300) CD16+ & CD56+: Normal range (300 to 1100)  
$P_1$: between patients before and control. $P_2$: between patients after and control. $P_3$: between patients before and after. 
$^*P>0.05$ is not significant.

Figure 2. Comparison of CD4+ cells and CD8+ cells between a neonatal case before phototherapy (a) and after phototherapy (b) and a control (c).
**Discussion**

Phototherapy has been effectively used as a relatively inexpensive and noninvasive method of treating neonatal hyperbilirubinemia. The decline in the number or exchange transfusions in recent years is, at least in part, likely a direct reflection of the effectiveness of phototherapy at treating hyperbilirubinemia (Maisels & Antony, 2008).

The effect of NNPT on immune regulation may partly be due to degrading bilirubin. Unconjugated bilirubin inhibits complement activation through the classical pathway (Basiglio et al., 2010) and prevents leukocyte migration (Keshavan et al., 2005). A proper increase in bilirubin levels during the neonatal period protects infants from oxidative stress and promotes Th2/Th1 switching, which prevents allergic manifestations in later periods of life. Thus, interfering with physiological bilirubin metabolism via NNPT may cause an immune system disorder (Gloria & Bottini, 2010).

This study shows that there is no significant difference in CD4⁺ and CD8⁺ lymphocyte level among patients before phototherapy compared to control group. And no significant difference in CD4⁺ and CD8⁺ lymphocyte level among patients 72 hrs after phototherapy compared to control group.

These results go in agreement with Karabayir et al., (2011) who studied the effect of phototherapy on the CD4⁺ and CD8⁺ lymphocyte level in newborn using 22 term neonates with indirect hyperbilirubinemia and 25 control term neonates without hyperbilirubinemia. He found that apart from significant increase was determined in CD4⁺ ratios after eight hours of the phototherapy. Non-significant change was determined in CD4⁺ and CD8⁺ lymphocyte level 48 hours after phototherapy.

Teunissen et al. (1993) investigated the consequences of direct exposure of T cells to low doses of ultraviolet B (UVB) *in vitro*. Compared with the unirradiated control, he found that 2 or 3 days after radiation exposure of graded single doses of UVB had no effect on CD4⁺ and CD8⁺ lymphocyte level.

However, McGrath et al. (1986) demonstrated that a significant decrease of the CD8 subset level occurs after UV irradiation.
in vitro or in vivo. And Hersey et al. (1983) found a decrease of the CD4+ cells. In an in vitro study, UV was reported to kill most of the T-cells in a dose dependent manner (Erduran et al., 1997). It is difficult to explain these differences, since the experimental designs are not comparable.

Also, this study shows that there is no significant difference in CD16+ & CD56+ lymphocyte levels among patients before phototherapy compared to control group. And no significant difference in CD16- & CD56+ lymphocyte levels among patients after phototherapy compared to control group.

These results go in agreement with a study performed by Neill et al., (1998) which reported that inpatients the levels of the CD16+ 56+ lymphocyte decreased after exposure to UV radiation but these findings were not significant.

These results also go in agreement with Kurt et al., (2009) who investigated the influence of the use of phototherapy in the treatment of neonatal hyperbilirubinemia on some lymphocyte subsets and cytokine Production. He found that all lymphocyte subsets were not statistically significant decreased by the 72 hrs of exposure to phototherapy, except the level of CD3+ lymphocyte subset was significantly lower in newborns at 72 hrs of exposure to phototherapy.

As well as Tobin et al., (2009) who stated that during and after narrow band ultraviolet B (UVB) treatment; there were no differences in lymphocyte subsets between blood samples of patients and controls.

In conclusion phototherapy has no significant effect on the level of CD4+, CD8+ and natural killer (CD16+ & CD65+) lymphocytes in full term neonates after 72 hrs of exposure.

References


