Effects of Phototherapy on Cytokines’ Levels and White Blood Cells in Term Neonate with Hyperbilirubinemia

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ABSTRACT

Objective: Phototherapy is the most common treatment used for severe jaundice. There is increasing evidence that phototherapy can directly affect the expression and function of cell surface receptors including adhesion molecules, cytokines, and growth factor receptors. The aim of this study is to investigate the effect of phototherapy use on the levels of interleukin (IL)-1α, IL-6, and tumor necrosis factor (TNF)-α as cytokine expressions from keratinocytes, and also white blood cell counts in the treatment of neonate with hyperbilirubinemia. Materials and Methods: We studied 32 term newborns with hyperbilirubinemia. Blood samples were obtained before and 72 h after phototherapy. Serum levels of IL-1α, IL-6, TNF-α, and WBC count were measured in the samples using appropriate methods. Results: Serum TNF-α at 72 h of exposure to phototherapy increased, while the levels of IL-1α and IL-6 at the same time were decreased. These changes were not statistically significant. WBC counts rose significantly with phototherapy. Conclusion: Phototherapy in term neonate does not affect cytokines’ levels, but can raise peripheral WBC count.

Key words: Cytokine, hyperbilirubinemia, phototherapy, term neonate

INTRODUCTION

Phototherapy is the most common treatment in use for severe jaundice.[1] It involves exposing the infant’s skin to light of specific wavelength (420–470 nm).[2] Some investigators suggest that this technique in vitro can induce breaking of DNA strands and sister chromatid mutations.[3,4]

There is increasing evidence that phototherapy can directly affect the expression and function of cell surface receptors including adhesion molecules, cytokines, and growth factor receptors.[5-7]

Phototherapy exerts actions on cellular element of peripheral part of immune system, as this part is readily accessible to photons. In addition to direct effect on peripheral immune system, photons have effects in the deeper part of immune system. For example, phototherapy can affect the synthesis and release of cytokines from peripheral immune system. Cytokines or soluble factors regulate or influence every aspect of immune cell growth, differentiation, and function. These factors, generally small, single chain peptides, are produced by cells of all types in the immune system, particularly by T cells and monocytes.[8-10] Interleukin (IL)-1 has extremely diverse biological activities. A prominent effect is enhancing the activation of B and T cells. It is a mediator of tissue inflammation, promotes prostaglandin synthesis, induces collagenase enzyme production, increases expression of adhesion molecules, and causes fever by acting on the central nervous system. It also influences the Langerhans cell function and is involved in the cutaneous response to phototherapy.[11,12]

IL-6 appears to act primarily as a differentiation factor that increases the production of antibodies by activated B cells. It increases platelet production and induces acute phase reactant protein production by liver cells.[12,13]

Tumor necrosis factor-a (TNF-α) serves as a co-stimulatory signal for activation and proliferation of B and T cells. It stimulates inflammation, fever, and acute phase reactants, and activates phagocytic cells. Corticosteroids inhibit TNF-α.[14] This factor is secreted in patients with infection or exposed to UV radiation.[14]
Most studies regarding the influence of phototherapy on the immune system have been done in laboratories and on animal subjects, while few studies have been performed in human subjects.

Sirota et al. observed that serum IL-β decreased but IL-2, IL-10, and TNF increased after phototherapy. In another study, Kurt showed that serum IL-8, IL-β, and TNF-α levels increased after exposure to phototherapy, whereas the level of IL-6 was not statistically altered.

We carried out this study to investigate the influence of phototherapy on the levels of TNF-α, IL-10, and IL-6 as cytokines expressed from keratinocytes, and also their effect on White Blood Cells (WBC) in the peripheral blood of babies with hyperbilirubinemia.

MATERIALS AND METHODS

This study was performed prospectively on 32 term newborn babies with hyperbilirubinemia in the newborn services at Amir Kola Hospital, Babol, Iran, in September 2009–January 2010, who were admitted due to hyperbilirubinemia. Parental informed consent was taken prior to inclusion and the study was approved by the local ethics committee. Inclusion criteria for this study group were cases of neonatal indirect hyperbilirubinemia where the use of phototherapy had begun according to the guidelines of the American Academy of Pediatrics. Exclusion criteria were premature babies, congenital malformations, congenital infections associated with TORCH, hypoxia, respiratory distress, neonatal hemolytic disease, sepsis, exchange transfusion, and also any surgical problems. In mothers, the exclusion criteria were diabetes mellitus, preeclampsia, steroid treatment, and use of drugs. Blood samples were obtained from hyperbilirubinemic term newborns before and at 72 h after exposure to phototherapy. Phototherapy was administered by five blue lights with wavelengths of 400–500 nm, placed 30 cm above the infants. Four milliliters of blood was obtained for cytokine analysis. The serum layer was separated by centrifugation and was frozen at –20°C.

Sampling was done by enzyme-linked immunosorbent assay (ELISA) for TNF-α, IL-10, and IL-6 levels with human cytokine kits (Bender Medsystems, A-1030 Vienna, Austria, and Europe). The assays were done according to the manufacturer’s instructions, and absorbance measurements were made on Stat Fox Microplate Reader. All measurements described above were carried out with kit control. One milliliter of blood was collected before and after phototherapy in glass tubes containing ethylene diamine tetraacetic acid. WBC count measurement with cell counter (ESI symex KX-21) was conducted in the laboratory.

Statistical analysis was performed using the SPSS 18.0 software for Windows XP. Paired-sample t-test procedure was used for the interpretation of the difference between WBC counts before and after exposure to phototherapy. The nonparametric Wilcoxon test was used for the interpretation of the difference of IL-1α, IL-6, and TNF-α before and at 72 h after exposure to phototherapy. P value less than 0.05 was considered to be significant.

RESULTS

Among the 32 neonates, 10 (31.3%) patients were girls and 22 (68.8%) were boys. Postnatal age was 3–19 days, with a mean of 6.3 days. Mean birth weight of newborns was 3.5 kg, with a range of 2.5–5.2 kg. Serum bilirubin levels were between 13 and 17.20 mg/dl with a mean of 15.9.

Mean serum TNF-α level before exposure to phototherapy was 12.95 pg/ml and after 72 h of phototherapy, it increased to 34.103 pg/ml, and this difference was not statistically significant (P=0.082). Also, mean serum IL-1α before and after phototherapy was 2.219 and 1.131 pg/ml, respectively, and this decreasing value was not significant (P=0.819) either. Mean IL-6 levels before and after phototherapy were 42.3 and 22.0 pg/ml, respectively, and this change was not significant (P=1.000).

WBC and all cytokine levels of the study are listed in Table 1. The mean of WBC count before and after phototherapy increased from 10.489.06 to 11.431.52, and was statistically significant (P=0.038).

DISCUSSION

Phototherapy is considered safe in neonates, with few side effects such as macular rash, hyperthermia, loose stool, and dehydration due to increased insensible water loss. Different studies have shown that cytokine production may be changed after exposure to phototherapy.

| Table 1: Statistics of cytokines and WBC before (B) and after (A) phototherapy |
|-----------------|-----|----------------|-------|---------|---------|---------|
|                 | n   | Mean           | Minimum | Maximum | P value |
| IL-1α           | (B) | 32             | 2.219   | 0.2     | 18.6    | 0.819 (IL-1α) |
| IL-1α           | (A) | 32             | 1.131   | 0.4     | 3.6     |          |
| IL-6            | (B) | 32             | 42.331  | 0.4     | 205.0   | 1.000 (IL-6) |
| IL-6            | (A) | 32             | 22.069  | 0.4     | 202.2   |          |
| IL-8            | (B) | 32             | 491.038 | 0.0     | 3016.0  | 0.750 (IL-8) |
| IL-8            | (A) | 32             | 338.825 | 0.0     | 2358.0  |          |
| WBC             | (B) | 32             | 10.489  | 5800    | 17.200  | 0.038 (WBC) |
| WBC             | (A) | 32             | 11.431  | 2500    | 20.700  |          |
| TNF-α           | (B) | 32             | 12.925  | 0.1     | 148.0   | 0.082 (TNF-α) |
| TNF-α           | (A) | 32             | 34.103  | 0.1     | 212.6   |          |
Sirota in 1999 examined the capacity of peripheral blood mononuclear cells to produce IL-1β, IL-2, IL-3, IL-6, IL-10, and TNF-α in 20 term newborns with jaundice after 24 h of exposure to phototherapy (wavelength 425–475 nm). Phototherapy induced a 70% increase in IL-2 secretion and 56% increase in IL-10 production, whereas the secretion of IL-1β was reduced by 43%. On the other hand, lipopolysaccharide induced TNF-α production was higher in the newborns by phototherapy. The synthesis of IL-3 and IL-6 did not change significantly. Phototherapy affects the function of the immune system in newborns by alterations in cytokine production.[11] Exposure to UV radiation results in the suppression of many cell-mediated immune responses.[18,19]

Despite the long-term use of phototherapy, the mechanisms of action are quite unclear. Many of its effects are certainly mediated by induction of apoptotic cell death and another major mechanism is the induction of immunosuppression.[20]

Kurt studied the level of cytokines in 21 newborns before and after 72 h of phototherapy and concluded that TNF-α, IL-8, and IL-1β levels increased after phototherapy, while the serum IL-6 level did not significantly change and the lymphocyte subset decreased.[13]

Mrkaić et al. observed that phototherapy may cause a disturbance in the behavior and higher incidence of infections in neonate. They examined the effects of phototherapy on the immune system of neonates without signs of infection, anoxia, or birth injury. Their results showed an increase in the total number of peripheral WBCs, polymorphonuclears, lymphocytes, and monocytes, as well as a delay in the chemiluminescence response of the peripheral blood phagocytes. They concluded phototherapy may complicate the existing infection, though those findings were temporary.[11]

Schwarz showed in 1987 that UV irradiation of epidermal cells in vitro and in vivo leads to an enhanced synthesis of the immunostimulating cytokine IL-1 and also UV exposure in vivo results in local as well as systemic immunosuppression. He concluded that epidermis may not only be considered as a simple barrier against harmful agents, but also represents an active element of the immune system.[9]

In different studies on cytokines' levels of subjects exposed to phototherapy, the results were not homogenous. The majority of investigators found that the levels of serum cytokines such as TNF, IL-6, and IL-1 were higher after phototherapy or UV exposure, while others found that these factors were lower or not changed. In the present study, levels of TNF-α were increased while the levels of IL-10 and IL-6 were decreased, but these changes were not statistically significant. The limitations of this study are duration of phototherapy, temperature for preservation, sample size, and kind of phototherapy.

In our study, the WBC count was increased by exposure to phototherapy, as the neonates in our study were healthy except being hyperbilirubinemic; increase in WBC may be due to stress of admission or beginning of infection, and increase in the total number of peripheral WBC itself is nonspecific. This observation requires further study.

**CONCLUSION**

The results demonstrate that phototherapy does not significantly affect cytokine levels in neonatal jaundice, while this therapy affects the number of peripheral WBC.

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