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RESEARCH ARTICLE

The Effect of Intensive Phototherapy Treatment on CD4 and CD8 T-Lymphocyte subsets in Neonatal Jaundice

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Abstract

Introduction: Jaundice is the most common condition that requires medical attention and hospital readmission in neonates. Serum bilirubin levels may rise excessively, which can be cause lifelong neurologic sequelae (kernicterus).

Objectives: To investigate the effect of intensive phototherapy on the expression of CD4 and CD8 lymphocyte subsets in neonates with indirect hyperbilirubinemia.

Patients and Methods: This is a prospective study included 30 full term neonates admitted to neonatal intensive care unit, Minia university hospital and 20 full term healthy neonates during the period from October 2016 to October 2017.

Results: There were no significant differences regarding CD4%, CD8% and CD4/CD8 ratios in neonates with indirect hyperbilirubinemia either before or after phototherapy. After phototherapy there were no significant differences between cases and controls as regarding CD4% and CD4/CD8 ratio while there was a significant decrease in CD% in cases compared to controls (p value 0.03). A significant positive correlation between CD4% and age (r- value 0.39, p-value 0.02) was present in cases.

Conclusions: The results demonstrated that in addition to the well-known positive effect of phototherapy on neonatal serum bilirubin level, this treatment have no effect on the function of the immune system in neonates as regards CD4 and CD8 subsets as well as CD4/CD8 ratio .

Key words: Phototherapy, Neonatal, Jaundice, CD4, CD8, T-Lymphocyte

Introduction:

Jaundice is the most common condition that requires medical attention and hospital readmission in neonates. The yellow coloration of the skin and sclera in neonates 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 rise excessively, which can be cause for concern because unconjugated bilirubin is neurotoxic and can cause death in neonates and lifelong neurologic sequelae in infants who survive (kernicterus) [1]. 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 neonates now Phototherapy is the current "drug" of choice to reduce the severity of neonatal unconjugated hyperbilirubinemia regardless of its etiology [2]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. In modern neonatal ICUs (NICUs) exchange transfusions are rare and are only used as a rescue therapy to avoid kernicterus in neonates with severe jaundice when phototherapy is inadequate [3]. Phototherapy is generally regarded as safe, but some investigators have expressed concern about its potential toxic effect [4].

Aspberg S et al., 2010 [5] reported an association between neonatal phototherapy and risk of childhood asthma in a large population-based study. He confirmed an association between some maternal and perinatal factors and childhood asthma, including neonatal phototherapy and/or icterus. Interestingly, a study including patients with more than a 30-years follow-up period has shown that neonatal phototherapy is associated with allergic rhinitis and conjunctivitis [6]. The evidence that neonatal phototherapy inhibits the immune system is supported by findings that neonatal phototherapy affects the Th-2/Th-1 switch, ultimately causing allergic diseases during childhood and later in life. There are a few possible mechanisms for the Th-2/Th-1 switch disorder [7]. The effect of neonatal phototherapy on immune regulation may partly be due to degrading bilirubin. Unconjugated bilirubin inhibits complement activation through the classical pathway [8] and prevents leukocyte migration [9]. 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 neonatal phototherapy may cause an immune system disorder [6]. During last few decades, some investigators have expressed concern about phototherapy potential toxic effects. One possible harmful consequence is affection of cytokines production and lymphocytes subtypes which can affect the function of the immune system in neonates [10]. Phototherapy increases DNA damage in lymphocytes of hyperbilirubinemic neonates [11].In some studies, levels of CD4 and CD8lymphocyte subsets are decreased after exposure to phototherapy but these findings were not significant. [10].

Patients and Methods:

This is a prospective study included 30 full term neonates admitted to neonatal intensive care unit, Minia university hospital and 20 full term healthy neonates during the period from October 2016 to October 2017. They were classified into two groups: Group I (Patients Group): thirty full term neonates with neonatal indirect hyperbilirubinemia in need for intensive phototherapy according to guidelines of American Academy of Pediatrics subcommittee on hyperbilirubinemia [2]. Group II (Control Group): twenty healthy full term neonates without jaundice.

Inclusion criteria

Full term neonates: 37-42 weeks Appropriate for gestational age. Indirect Hyperbilirubinemia in need of intensive phototherapy according to guidelines of American Academy of Pediatrics subcommittee on hyperbilirubinemia [2]. Initiation of phototherapy during the first week of life.

Exclusion criteria

Preterm neonates: less than 37 weeks, Post term neonates: 42 weeks or more, Small for gestational age and large for gestational age. Neonates with direct hyperbilirubinemia (cholestasis) or suffering from any systemic illness other than neonatal jaundice (i.e. infant of diabetic mother, congenital pneumonia, congenital infections and birth asphyxia, sepsis . Neonates suffering from congenital anomalies) or those beyond the first week of life .

Methods

For patients and control groups the following were done : Full history taking : include

Antenatal history excluding the previously mentioned exclusion criteria.Natal history including mode of delivery, gestational age.

Postnatal history including onset of jaundice, pallor, and cyanosis. Full clinical examination: Apgar score. Assessment of gestational age .

Birth weight and length.Obvious congenital anomalies. Systemic examination: Infants with jaundice examined for the following physical findings: Extravasated blood, pallor, plethora, petechiae, hepatosplenomegaly, signs of neonatal sepsis, signs of bilirubin encephalopathy.

Study Design

Patients group received phototherapy through intensive phototherapy unit (Fanem Bilitron Bed 4006. São Paulo - SP – Brasil). Which consisted of 17 Super LED`s on the blue spectrum range, focused in 450 nm, with irradiance 47.5 ±5 µW/cm2 Nm (average at the center of the mattress) and dimensions of the light stain over the mattress: 32 x 20 cm .

Intensive Phototherapy was applied to jaundiced neonates for 6 hours with all precautions needed for protection of neonates against the harmful effect of phototherapy applied according to A.A.P 2004 Guidelines as: Covering of his eyes and genitalia, Continous feeding on demands and regular measurement of bilirubin level .

Laboratory investigations

Total and direct serum, Bilirubin.Haemoglobin.

Total leukocytic count ,Platelets count. Serum CD4 and CD8 levels before and 6-8 hours after intensive phototherapy.

Blood sampling

Blood samples were collected from patients before phototherapy and 6-8 hours after exposure to intensive phototherapy, and samples were collected from controls at time of examination.

Blood specimen

The peripheral blood in a sterile tube with an anticoagulant (Heparin or EDTA), stored at room temperature and stained within 48 hours of drawing.

Principle for CD4 and CD8 measurements

This test is based on the specific binding of monoclonal antibodies to the antigenic determinants expressed on the surface of leukocytes. The monoclonal antibodies are labeled with different fluorochromes which are excited via laser beam from a flow cytometer during analysis. Subsequent emissions of light from the fluorochromes of each cell are collected and analyzed by a flow cytometer. The fluorescence intensity differences enabled the separation of cell subsets based on the expression of analyzed antigens. The specific staining of blood cells was performed by the incubation of blood samples with the reagent followed by a lysis of red blood cells. Afterwards, unaffected leukocytes were subjected to analysis by a flow cytometer.

We analyzed the sample immediately using a flow cytometer.

Results:

There were no significant differences between cases and controls as regarding age, sex, birth weight, gestational age, mode of delivery and maternal age(Table 1) .there is no statistically significant differences between cases and control as regarding hemoglobin, platelets and total leucocytic count(Table 2). there is statistically significant differences between cases and control as regarding total and direct bilirubin (p value <0.001) (Table 3).There were no significant differences regarding CD4%, CD8% and CD4/CD8 ratios in neonates with indirect hyperbilirubinemia either before or after phototherapy (Table4) and (Table5). After phototherapy there were no significant differences between cases and controls as regarding CD4% and CD4/CD8 ratio while there was a significant decrease in CD% in cases compared to controls (p value 0.03) (Figure1) and (Figure 2). A significant positive correlation between CD4% and age (r-value 0.39, p-value 0.02) was present in cases. The study was approved by the local research ethics committee of the faculty of medicine in Minia University and written informed consents were obtained from the parents of all neonates to share in the study.

Discussion:

Neonatal jaundice is one of the most common conditions confronting neonatologists daily. About 60% of term and 80% of preterm infants develop jaundice in the first week of life [12].

Fortunately, a noninvasive and easily available treatment, neonatal phototherapy (NNPT), is effective in degrading unconjugated bilirubin. Following the discovery of NNPT in the 1950s, many clinical trials have addressed its application to neonatal jaundice [13]. In 1985, the National Institute of Child Health and Human Development reported that NNPT was as effective as exchange transfusion in preventing neurological sequelae [14]. Since then, NNPT has been widely adopted as the initial therapy of choice for hyperbilirubinemia. When comparing blue, blue-green, green, and white light, researchers found that blue light was the most effective in degrading bilirubin [15]

Therefore, NNPT with blue light is generally used in the clinical practice. The spectrum (380–550 nm) of blue light consists mainly of visible light with a peak at 450 nm and a minor component of ultraviolet light. NNPT reduces serum bilirubin levels by converting bilirubin through structural photoisomerization and photooxidation into excretable products [16].

Both intensive and conventional therapy causes endogenous mononuclear leucocyte DNA damage in the same way and same percentage in

jaundiced term infants; and thus it might affect the immune system [17].

The aim of this study was to investigate the effect of intensive phototherapy used for treatment of neonatal hyperbilirubinemia on lymphocyte subsets CD4 and CD8 as a reflection of the immune status of the neonates.

Comparison of the demographic characteristics between patients and controls showed no statistically significant differences between patients and controls as regarding age, sex, birth weight, gestational age, mode of delivery and maternal age to eliminate their effects on the CD4 and CD8 subsets. Also, our results showed that there are no significant differences in hemoglobin, platelets levels and total leucocytic count between patients and control group as the bilirubin itself had no effect on hemoglobin, platelets and total leukocytic count.

As expected the results of this study showed that there is highly significant difference as regarding total and direct bilirubin between patients and control group as they were higher in patients group. This study showed that there is no significant difference in CD4, CD8 and CD4/CD8 ratio among patients before phototherapy compared to control group.

These results go in agreement with [18] who found no significant effects of indirect hyperbilirubinemia on CD4 and CD8 lymphocyte level. Also, there is no significant difference in CD4 and CD8 level and among patients before and after phototherapy.

Our results agreed with a study [19] found no statistically significant difference between lymphocytes subsets before and after 72 h of exposure to phototherapy. Moreover, our results were in agreement with[10] who found that all lymphocyte subsets were not significantly decreased by the 72 h of exposure to phototherapy, except for the percentage of CD3 lymphocyte subset which was significantly lower in new-borns at 72 h of exposure to phototherapy .

Karabayir et al, 2011 [18] 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 ($p< 0.05$). Non-significant change was determined in CD4 and CD8 lymphocyte level 48 hours after phototherapy ($p > 0.05$) .This difference between our results and karabayir may be due to time of measurement for CD4 and CD8 after phototherapy .

Teunissen et al 1993 [20]investigated the consequences of direct exposure of T cells to low doses of ultraviolet in vitro and he found

that 2 or 3 days after radiation exposure of graded single doses of UV had no effect on CD4 and CD8 lymphocyte level as well as with other reports [21] who stated that during and after ultraviolet treatment, there were no differences in lymphocyte subsets between blood samples of patients and controls.

In our study on comparing the ratio between CD4/CD8 ratio among the studied groups and among patients before and after exposure to phototherapy there was no statistical difference. These results concurred with [18]. who also found that there was no change in CD4/CD8 ratio among patients or among the studied groups . This study showed that there is no significant difference in CD4 level and CD4/CD8 ratio among patients after phototherapy compared to control group but there is significant difference in CD8 level among patients after phototherapy compared to control group.

In contrary to our results, early studies[22] demonstrated that a significant decrease of the CD8 subset level occurs after UV irradiation in vitro or in vivo, and [23] 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 [24]. It is difficult to explain these differences, since the experimental designs are not comparable as well as safety of wave length of phototherapy. In this study we didn't find correlation between CD4 level of patients and other parameters (birth weight, gestational age, age of the mother, hemoglobin, platelets, total leukocytic count, total and direct bilirubin). There is significant positive correlation between CD4% before phototherapy and age, this result need a larger number of neonates for explanation. Also, we didn't find correlation between CD8 level of patients and other parameters (age, birth weight, gestational age, Age of the mother, hemoglobin, Platelets, total leukocytic count, Total and direct bilirubin).These results are in agreement with studies done by [10, 18 and 25] who reported similar results .

Conclusions:

The results demonstrated that in addition to the well-known positive effect of phototherapy on neonatal serum bilirubin level, this treatment have no effect on the function of the immune system in neonates as regards CD4 and CD8 subsets as well as CD4/CD8 ratio .

Conflict of interest: The authors declared no conflict of interest.

Author's contributions: EA and AA conceived the study. HM revised the patients' medical reports and the final manuscript. All authors revised the final draft of the manuscript.

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