Effect of Intensive Phototherapy on T and B Lymphocyte Function in Neonatal Jaundice

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Abstract

Background: Jaundice is one of the most common problems that affect newborns in the first few days of life. Approximately 60% of full-term and 80% of the preterm newborn may develop neonatal jaundice. Although neonatal jaundice is of physiological type in most of these cases, some cases may have elevated serum bilirubin levels which, if not treated, may lead to bilirubin encephalopathy and subsequently bilirubin induced neurological dysfunction (BIND).

Objective: This study aimed to evaluate the effect of intensive phototherapy on B and T cells by measuring the percentages of CD19+, CD4+ and CD8+ lymphocytes subsets in newborns after 72 hours from exposure to intensive phototherapy to evaluate its effect on the immune system.

Patients and methods: A prospective cohort study carried out in Zagazig University Hospital, Pediatric Neonatal Intensive Care Unit (NICU) and Clinical Pathology Departments. The study included 50 full-term newborns. **Results:** There was no significant difference regarding CD4, CD8 and CD19 for the patient group. Furthermore, the study showed that the percentages of CD4+ and CD8+ lymphocytes subsets showed no change in newborns after 72 hours of exposure to phototherapy, but CD19+ was highly significantly lower before treatment only. In addition, the follow up of those 25 jaundiced newborns for occurrence and frequency of infection and need for hospitalization for a period of six months after discharge showed no effect.

Conclusion: our results showed no effect of phototherapy on lymphocyte subsets after 72 hours of exposure and no effect on infant immunity.

Keywords: Intensive phototherapy**,** Neonatal Jaundice, T and B Lymphocyte.

Introduction:

Neonatal jaundice is one of the most common conditions challenging neonatologists daily. Yellow discoloration of a newborn baby's skin and eyes caused by hyperbilirubinemia resulted from an accumulation of unconjugated bilirubin[(1)]. Although most neonatal hyperbilirubinemia cases are physiological and reflect a normal transitional phenomenon, excessive unconjugated bilirubin is a potential neurotoxin. In some infants, hyperbilirubinemia can become dangerous where serum bilirubin levels may rise excessively and cause death in neonates and lifelong neurologic sequelae in infants who survive (kernicterus) [(2)].

Total bilirubin (TB) levels may rise and exceed the physiological limit, rarely to very high levels and cause kernicterus. Infant jaundice usually occurs because a baby's liver isn't mature enough to eliminate excessive bilirubin levels in the bloodstream. About 60% of term and 80% of preterm infants develop jaundice in the first week of life. In some babies, an underlying disease may cause infant jaundice. Particularly in the presence of certain risk factors for severe jaundice, infants should be monitored to identify those who may develop severe hyperbilirubinemia. Besides, in rare cases, acute bilirubin encephalopathy or kernicterus necessitates a rapid intervention strategy. Phototherapy is the most widely used therapy for neonatal jaundice management[(3,4)].

Neonatal phototherapy (NNPT), a non-invasive, readily available therapy, has been widely used to treat neonatal for more than half a century. It effectively decreases or prevents the rise of serum unconjugated bilirubin levels and reduces the need for exchange transfusion in neonates[(5)]. Fundamentally, phototherapy uses light energy to treat severe hyperbilirubinemia in both; term and large preterm infants within the neonatal period. Technically, exposing the visible light will cause changes to the molecular structure of bilirubin. Thus, resultant products are less lipophilic than bilirubin and can be excreted in bile or urine without conjugation[(2,6)].

The effectiveness of phototherapy is dependent on the intensity and wavelength of used light. There is no standardized method of delivering phototherapy, and its units vary widely; several phototherapy devices are available, which use light sources with different wavelengths and densities. Current phototherapy systems use conventional compact fluorescent lamps or light-emitting diodes (LEDs), of which LEDs generate significantly higher light irradiance levels[(5)].

Although phototherapy is currently available and generally considered safe and well-tolerated in neonatal jaundice treatment due to its efficacy and apparent safety, concerns about the potentially toxic effects of phototherapy have been expressed. Some reports indicated an increase in mortality in low birth weight (ELBW) infants exposed to prolonged phototherapy (PT) had raised questions about its safety.

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Also, recent studies showed phototherapy might cause oxidative stress, lipid peroxidation, and DNA damage [3,7].

This study aimed to evaluate the effect of intensive phototherapy on T and B lymphocyte function throughout the treatment of neonatal jaundice and the correlation before, during and after phototherapy treatment by assessing the impact of risk factors of intensive phototherapy and measuring the percentage of CD4, CD8 and CD19 subsets.

Patients and methods:

A prospective cohort study was carried out in Zagazig University Hospital, Pediatric (NICU) and Clinical Pathology Departments from June 2021 to December 2021. The study included 50 full-term newborns who were divided into 2 groups of age and matched sex: Group I: Twenty-five matched healthy full-term newborns without neonatal jaundice nor received phototherapy. Group II: Twenty-five full-term newborns with neonatal indirect hyperbilirubinemia treated by intensive phototherapy for an average of 3 days.

Ethical considerations:

Written informed consent was obtained from all participants' parents and the study was approved by the Research Ethical Committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria: Both males and females. Full-term neonates with bodyweight > 2.5 kg. Neonate's age ranges from 1 to 28 days old at the time of admission with indirect hyperbilirubinemia in need of phototherapy. Postnatal age: less than or equal to 14 days, with neonatal indirect hyperbilirubinemia and treated by conventional phototherapy based on American Academy of Pediatrics recommendations[8]. Patients Group: full-term newborn of gestational age of more than 37 weeks. Untreated control group: healthy full-term newborn without neonatal jaundice both clinical and laboratory.

Exclusion criteria: Preterm neonates. Full term ≤ 2.5 kg in weight. Neonates suffering from sepsis or any congenital anomalies. Neonates with hypoxic-ischemic encephalopathy. Neonates in need of exchange transfusion. Neonates suffer from any congenital anomalies.

All patients were subjected to full history taking, full clinical examination. Phototherapy was carried out for the patients' group as the infants were uncovered except for shielded genitalia and eyes. White fluorescent lamps emit light at a wavelength of 420-470-nm, placed at a 40 cm distance from the neonates.

Routine Laboratory Investigations: total and direct serum bilirubin measured spectrophotometrically using automated analyzer "Roche Cobas 8000-c702" (Roche Diagnostics, Germany). CBC by automated cell counter "Sysmex XS" (Sysmex Corporation, Japan). The blood group of both; patients and mothers.

Special Laboratory Investigations: Plasma lymphocytes% of CD4+, CD8+ and CD19+ by flow cytometry. CD4 PE Anti Human CD4 Antibody. CD8 FITC Anti Human CD8 Antibody [OKT-8] E-AB-F1110D. Anti-Human CD19 Monoclonal antibody per CP Conjugated [CB+9] E-AB[1].

Follow up:

Clinical follow up of patients group for a period of six months after phototherapy to monitor any incidence, frequency of infection or need for hospitalization. Some patients had upper respiratory tract infections in the form of fever, cough, and runny nose, while some patients had gastroenteritis in the form of diarrhea. Most respiratory virus infections in early childhood are confined to the upper respiratory tract, where upper respiratory tract infection (URTI) in infants may lead to lethargy and poor feeding. Infective gastroenteritis in young children is characterized by the sudden onset of diarrhea, with or without vomiting. Most cases are due to a viral infection, but bacterial or protozoal infections cause some. The illness usually resolves without treatment within days, but severe diarrhea can rapidly cause dehydration.

Statistical Analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Wilk test. Qualitative data were represented as frequencies and relative percentages and were compared by Chi square test (χ^2). Quantitative data were expressed as mean ± SD (Standard deviation), median and range. Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric) and Mann-Whitney test was used to compare abnormally distributed variables (Nonparametric). P value < 0.05 was considered significant and <0.001 was considered highly significant.

RESULTS

Table 1 shows that there was no statistically significant difference regarding gestational age, weight, head circumference and sex between control and patient groups.

Table (1): Basic demographic data distribution among studied groups

			Control Group	Patient Group	P
Gestational age (weeks), range			37- 40	37- 40	-
Weight (kg), Mean±SD			3.37±0.32	3.15±0.36	0.358
Head circumference (cm), Mean±SD			32.56±1.32	31.92±1.22	0.082
Sex	**Male**	**N**	11	13	0.52
		%	44.0%	52.0%	-
	Female	**N**	14	12	0.57
		%	56.0%	48.0%	-
Total		**N**	25	25	-

Table 2 shows a statistically significant difference regarding total and indirect bilirubin before and after treatment between both groups. There was also a statistically significant difference regarding total and indirect bilirubin before and after treatment in the patient group.

Table (2): Bilirubin level among studied groups before and after 72 hours of treatment

	Control Group Mean±SD	Patient Group Mean±SD	P
T bilirubin [before] (mg/dL)	1.74±0.34	17.74±3.17	<0.01*
T bilirubin [after] (mg/dL)	1.74±0.34	8.10±2.06	<0.01*
P		<0.01*	
Indirect bilirubin [before] (mg/dL)	1.07±0.15	16.43±4.02	<0.01*
Indirect bilirubin [after] (mg/dL)	1.07±0.15	6.30±1.08	<0.01*
P		<0.01*	

*: Significant

Table 3 shows that patients group had significantly lower value of CD8 before treatment compared to control group. The patient group significantly increased after treatment regarding both of CD8 and CD19 compared to before treatment values.

Table (3): Comparison of percent of CD4, CD8, and CD19 before and after 72 hours of treatment among studied groups

	Control Group Median (range)	Patient Group Median (range)	P
CD4-before	15.30 ±3	13.85 ±2	0.085
CD4- after	15.30 ±3	16.10 ±4	0.265
P	-	0.058	-
CD8-before	12.20 ±3	7.90 ±1	0.032*
CD8-after	12.20 ±2	10.85 ±2	0.096
P		0.042*	
CD19-before		8.60	2.27
CD19-after		8.60	7.85
P		<0.01*	

*: Significant

Table 4 shows that there was no significant difference between groups; also, there was no significant change among patients between before and after treatment as regard CD4/CD8 ratio.

Table (4): CD4/CD8 ratio before and after 72 hours of treatment among studied groups

	Control Group Median (range)	Patient Group Median (range)	P
CD4/CD8 before	1.10 ±0.2	1.52±0.2	0.228
CD4/CD8 after	1.10 ±0.2	1.63 ±0.2	0.079
P		0.236	

Table 5 shows that o significant correlation was detected between TSB and each of CD4 or CD8, but there was a significant negative correlation between CD19 before and after treatment and TSB.

Table (5): Correlation between total serum bilirubin (TSB) and percentages of CD4, CD8 and CD19 before and after 72 hours of treatment

		Total bilirubin before	Total bilirubin after
CD4-before	**R**	0.043±0.001	-0.138±0.002
	P	0.837	0.512
CD4-after	**R**	0.093±0.003	0.082±0.004
	P	0.657	0.696
CD8-before	**R**	0.207±0.03	0.025±0.03
	P	0.321	0.905
CD8-after	**R**	0.053±0.003	-0.055±0.002
	P	0.801	0.794
CD19-before	**R**	-0.434±0.1	-0.488±0,1
	P	0.032*	0.018*
CD19-fter	**R**	-0.469±0.1	-0.511±0.1
	P	0.008*	0.001**

R: Correlation coefficient, *: Significant, **: Highly significant

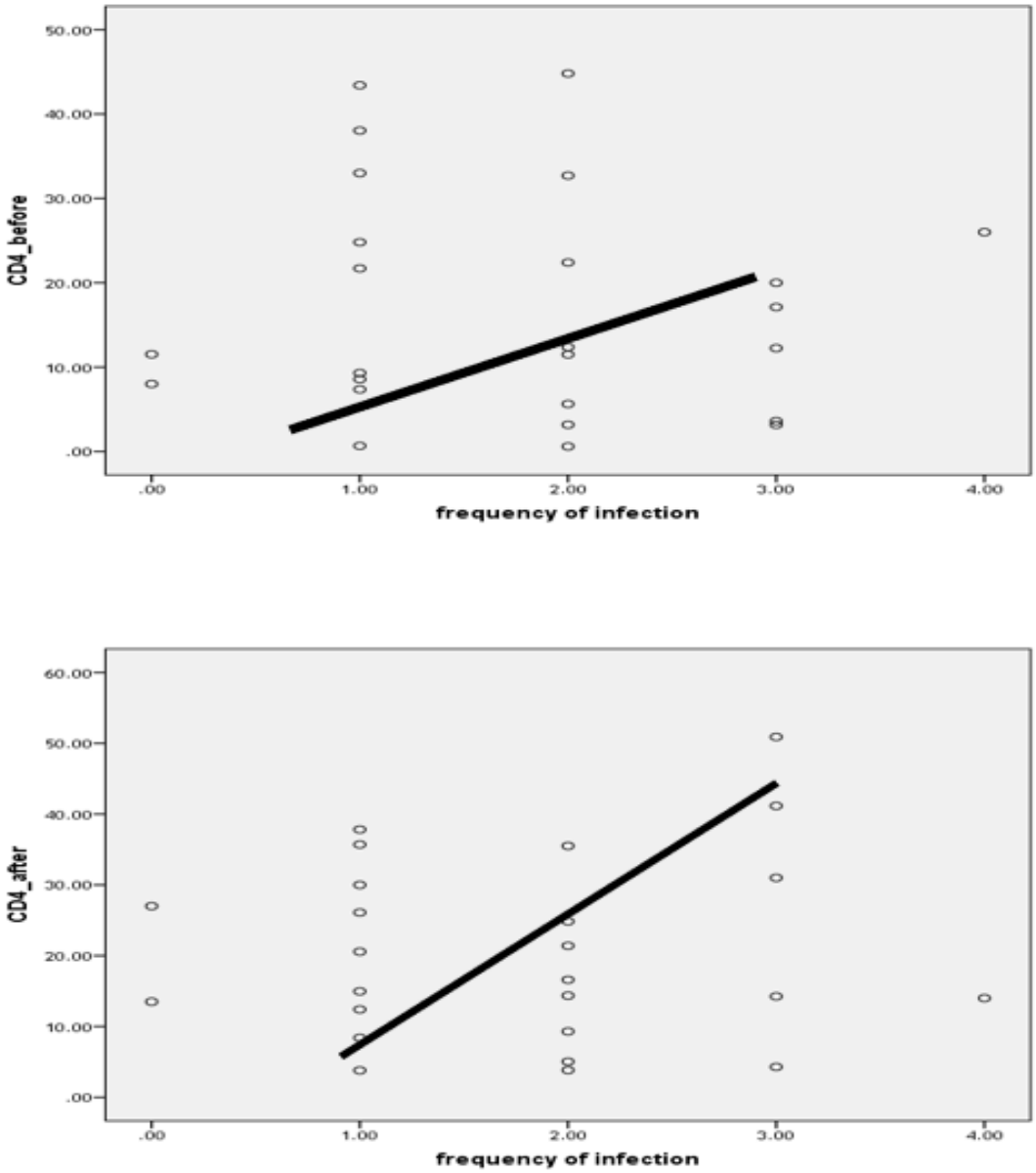

Figure (1): Positive correlation between times of infection and value of CD4 before and after treatment.

Table 6 shows that CD4 and CD8 were significantly higher among cases who had infection.

Table (6): Comparison of percent of CD

	Frequency of Infection Median	Infection Median	P
CD4-before	9.76	17.48	0.041*
CD4-after	20.25	20.70	0.963
CD8-before	5.11	9.57	0.047*
CD8-after	8.87	10.32	0.353
CD19-before	3.27	2.86	0.293
CD19-after	5.76	6.26	0.144

*: Significant

DISCUSSION

The demographic characteristics between patients and controls showed no statistically significant differences regarding gestational age, sex, and birth weight. These results agree with **Karabayir *et al.*[9] and Yahia *et al.*[10]**.

In our study, a comparison between patients' total serum bilirubin before and after phototherapy showed a statistically significant difference, meaning that phototherapy has a positive effect on neonatal hyperbilirubinemia. These results go in agreement with **Yahia *et al.*[10] and Shapiro and Riordan[11],** who found that ultraviolet phototherapy is a potent and effective treatment for neonatal jaundice.

This study showed that the percentages of CD4+ and CD8+ lymphocytes subsets showed no change in newborns after 72 hours of exposure to phototherapy, but regarding CD19 (B lymphocyte) in our study, there was statistically significant lower CD19 in patient group in comparison to control group before phototherapy but after phototherapy there was no statistically significant difference.

In our study, upon comparing patients before phototherapy and untreated control group as regard CD19+, CD4+ and CD8+ lymphocyte percentage, there was a statistical difference only in CD19+ lymphocyte percentage. Although CD19 lymphocytes percentage was significantly higher in the untreated control group than patients before phototherapy, there was no significant difference in CD19 between patients before and after 72 hours (h). But still, there was an increase in the percentage of CD19 lymphocytes 72 h after phototherapy, which would be closer to the untreated control group. This increase was highly related to the TSB level, where a decrease in TSB level led to an increase in CD19+lymphocytes percentage. These results were in line with **Ventura *et al.* [12]**, who demonstrated that unconjugated hyperbilirubinemia has an inhibitory effect on CD19 B cells, resulting from the induction of necrosis apoptosis in mature immune cells.

In the present study, there was a significant correlation between total serum bilirubin level and CD19+lymphocyte % before and after phototherapy. These results are in line with **Maharoof *et al.* [13],** who demonstrated that unconjugated hyperbilirubinemia has an inhibitory effect on CD19 B cells, which could result from induction of necrosis apoptosis in mature immune cells.

Our results agree with **Rashedy *et al.*[14],** who studied the effect of phototherapy on some lymphocytes subsets (CD4, CD8, CD19) in 30 term neonates with indirect hyperbilirubinemia and 25 healthy term neonates as a control group. They found no statistically significant difference between lymphocytes subsets before and after 72 hours of exposure to phototherapy.

These results agree with studies done by **Karabayir *et al.*[9] and Ebbesen *et al.*[15]**, who reported similar results. Moreover, our results agreed with **Rashedy *et al.*[14]** who found that all lymphocyte subsets were not statistically significantly decreased by the 72 h of exposure to phototherapy, except for the percentage of T lymphocyte subset, which was considerably lower in newborns at 72h of exposure to phototherapy. **Karabayir *et al.* [9]** noticed a significant increase in CD4+ rate after eight hours of phototherapy. However, there was no significant change in lymphocyte subsets 48 h after phototherapy.

There was no statistical difference in our study on comparing the ratio between CD4/CD8 ratio among the studied groups and patients before and after exposure to phototherapy. These results concurred with **Karabayir *et al.* [9]**, who also found no change in CD4/CD8 ratio among patients or the studied groups. This study showed no significant difference in CD4 level and CD4/CD8 ratio among patients after phototherapy compared to control group but there was a significant difference in CD8 level among patients after phototherapy compared to the control group.

El-Mazary *et al.* [1] studied the effect of phototherapy on the lymphocyte subsets in newborns among 22 term neonates with indirect hyperbilirubinemia and 25 control term neonates without hyperbilirubinemia. He noticed a significant increase in CD4+ % after eight hours of the phototherapy ($p< 0.05$). There was a non-significant change in lymphocyte subsets 48 hours after phototherapy ($p> 0.05$).

In our study, regarding the relation between frequency of infection and CD: Only CD4 and CD8 were significantly higher among cases that had an infection (positive correlation between them).

In addition, in our study, the follow up of patients for six months after their discharge showed no increase in the frequency of infections or need for hospitalization. This does not agree with **Usatin *et al.*** [16], who studied the effect of neonatal jaundice and phototherapy on the frequency of first-year outpatient visits and found that there was a slight increase in first-year outpatient visits rates.

CONCLUSION

In conclusion, our results showed no effect of phototherapy on lymphocyte subsets after 72 hours of exposure. Also, there was no correlation between exposure to phototherapy and occurrence and frequency of infection or admission to hospital. In addition, more studies are needed to determine the effect of intensive phototherapy on the immune system. Follow up for occurrence and frequency of infection for a period of more than six months may be needed.

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