The Effect of Phototherapy on the Lymphocyte Subsets in Newborn

Yenidoğanda Fototerapinin Lenfosit Alt Grupları Üzerine Etkisi

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ABSTRACT Objective: The purpose of our study is to investigate the effect of phototherapy on lymphocyte subsets in the treatment of hyperbilirubinemia in neonates. **Material and Methods:** Twenty two term infants as the study group and 25 term infants as the control group were hospitalized. Lymphocyte subsets levels were investigated in blood samples during hospitalization in all infants. However, subsets levels were investigated before and eight hours after the phototherapy, and once again after 48 hours in 16 infants in the study group. **Results:** The lymphocyte count of infants in the study and control groups were found similar, as well as the lymphocyte subsets ratios. Apart from CD4+ lymphocyte count of the study group eight hours after the phototherapy, any significant change was not observed in lymphocyte subsets. However, significant increase was determined in CD4+ ratios after phototherapy ($p< 0.05$). Any significant change was not determined in lymphocyte count and lymphocyte subsets 48 hours after phototherapy ($p> 0.05$). **Conclusion:** Apart from CD4+ count eight hours after phototherapy, any effect was not determined on the whole T-lymphocyte profile after 48 hours. Therefore, we assume that the possible effects of phototherapy on the immune system is not directly related to the effects on T-lymphocytes. The increase in CD4+ which appears eight hours after phototherapy requires more detailed studies regarding the effect of phototherapy on the immune system.

Key Words: Hyperbilirubinemia, neonatal; lymphocytes; infant, newborn

ÖZET Amaç: Çalışmamızın amacı yenidoğanda hiperbilirubinemi tedavisinde uygulanan fototerapinin lenfosit altgrupları üzerine etkisinin araştırılmasıdır. **Gereç ve Yöntemler:** Fototerapi almak üzere yatırılan 22 term bebek çalışma grubuna, 25 term bebek ise kontrol grubuna alındı. Çalışma grubundaki bebeklerin tümünden yatışta ve fototerapiden 8 saat sonra, 16 bebekten ise 48 saat sonra tekrar alınan, kontrol grubundaki bebeklerden ise yatış sırasında alınan kan örneklerinde lenfosit alt grupları çalışıldı. **Bulgular:** Çalışma ve kontrol grubu lenfosit sayıları ile lenfosit alt grupları oranları benzer bulundu. Çalışma grubunda fototerapiden 8 saat sonra lenfosit sayıları ile CD4+ dışında lenfosit alt gruplarında anlamlı değişiklik gözlenmedi. CD4+ oranında ise fototerapi sonrası anlamlı derecede artış saptandı ($p: 0.007$). Fototerapiden 48 saat sonra bakılan lenfosit sayıları ve lenfosit alt gruplarında ise fototerapi öncesi değerlerler karşılaştırıldığında anlamlı değişiklik saptanmadı ($p> 0.05$). **Sonuç:** Yenidoğanda hiperbilirubinemi nedeniyle uygulanan fototerapinin erken dönemde CD4+ dışında, 48 saat sonra ise tüm lenfosit alt gruplarının üzerine etkisi saptanmamıştır. Bu nedenle geç dönemde fototerapinin immun sistem üzerine olası etkileri T lenfositler ile direkt olarak ilişkili değildir. Fototerapiden kısa süre sonra ortaya çıkan CD4+ artışının immun sistem üzerine etkisi hakkında ise daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Hiperbilirubinemi; neonatal; lenfositler; bebek; yenidoğan

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Phototherapy of 425-475 nm wavelengths which is applied in the treatment of hyperbilirubinemia has been suggested to have potential side effects other than rash, diarrhea and insensible fluid loss, retinal

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damage and genetic defects.[1] In in-vitro studies, DNA breakages, changes in sister chromosomes and mutations are suggested to appear in cells exposed to light.[2,3] Although a great amount of ultraviolet (UV) light is absorbed in the epidermis, a small but important part of it passes through the epidermis and reaches the dermis. In this way, T-lymphocytes located around the capillaries of the papillary dermis may be exposed to low dose UV light as T-lymphocytes in the postcapillary venules of the papillary dermis.[4,5] Its effects on immune system by cytokines are generally investigated in in-vitro and in-vivo studies to determine the effects of UV on the immune system.[3,4,6,7] The aim of this study is to investigate the effects of phototherapy used in the treatment of hyperbilirubinemia on lymphocyte subgroups.

MATERIAL AND METHODS

A total of 47 term neonates, 22 as the study group and 25 as the control group, were included in the study. The study group was selected from the neonates hospitalized for phototherapy. However, the control group was selected from the neonates hospitalized due to a reason other than jaundice with the proper criteria. The exclusion criteria were determined as follows; total bilirubin level of >20 mg/dl, prematurity, sepsis, congenital abnormalies, elevation in direct bilirubin level, history of immune deficiency in the family, and the history of preeclampsia or any other diseases or drug use of the mother. Gestational age was assigned with the last menstruation date (LMD) when it was known, and with the Ballard scoring system when the LMD was not known. Neonates with a gestational week higher than 37 weeks were included in the study. Informed consents were obtained from the families. Blood samples of all neonates in the study group were drawn before and eight hours after the phototherapy, and also 48 hours after phototherapy in 16 cases. However, blood samples of the control group were drawn at the beginning of the hospitalization. Phototherapy lamp was located 30 cm above the patient who were uncovered except for shielded genitalia and eyes with AMS Phototheraphy System that contain six white fluorescent lamps emitting light at a wavelenght of 430-470 nm (intensity 12-16 μW/cm^2/nm).

Lymphocyte subtypes were studied using flowcytometry method (FACS Calibur, Becton-Dickinson Immunocytometry System, USA) from 2 cc blood samples stored in ethylenediaminetetracetic acid containing tubes in 48 hours following the drawing of blood samples. The sample tested in a laboratory managed in accordance with ISO 9001: 2000 quality standards.

Ethical approval was given by local ethical committee.

Statistical analyses were performed using the SSPS 10.0 for Windows. The data were expressed as means and standard deviations. The results of study group before and after phototherapy were compared using the paired-t test. In addition, the results of study and control groups were compared using the independent-t test. P values smaller than 0.05 were accepted as statistically significant.

RESULTS

The study and the control groups were similar with regard to gestational age and birth weight (Table 1). Lymphocyte counts and subgroup ratios are displayed in Table 2. Lymphocyte counts and subgroup rates before phototherapy were similar to the control group (Table 2) (p> 0.05). Although there was an increase in lymphocyte subgroups eight hours after the period of phototherapy when compared to the values before phototherapy, this increase was not statistically significant, except for CD4+ subgroups (Table 3). There was a statistically significant increase in CD4+ subgroups eight hours later

TABLE 1: Demographic characteristics of study and control groups.

	Study group	Control group	p
Gender female (n, %)	10 (45.4)	12 (0.48)	0.27
male (n, %)	12 (54.6)	13 (0.52)	0.37
Gestational age (wk)	38 ± 0.1	37.3 ± 0.2	0.13
Birth weight, (gm)	2819 ± 447	3120 ± 719.9	0.06
Blood bilirubine level at admission (mg/dl),	17.02 ± 2.38	(10.5-20.0)	
Phototheraphy starting time (hours),	146.58 ± 95.83		
Durations of phototheraphy (hours),	28.30 ± 13.49		

TABLE 2: Lymphocyte subsets of study and control group.

	Study group	Control group	P
Lymphocyte counts	4766.66 ± 1911	4044.02 ± 1418	0.31
CD3 +, %	75.10 ± 4.79	72.06 ± 7.46	0.06
CD19+, %	17.62 ± 3.5	17.69 ± 4.04	0.86
CD4 +,%	43.56 ± 7.39	40.14 ± 6.74	0.98
CD8 +,%	35.60 ± 6.28	32.42 ± 9.24	0.14
CD4 +/CD8 +	1.25 ± 0.16	1.19 ± 0.15	0.34
CD16-56 +, %	20.3 ± 4.30	19.35 ± 5.20	0.48
Activity	11.73 ± 1.89	12.73 ± 2.29	0.10
CD45 +, %	93.07 ± 1.20	93.08 ± 1.91	0.98

TABLE 3: Lymphocyte counts and subgroup rates before and after 8 hours phototherapy.

	Before Phototheraphy	After 8 hours phototheraphy	p
Lymphocyte counts	4766.66 ±1911	4766.66± 1607	0.444
CD3 +, %	75.10 ±4.79	77.23 ± 4.66	0.059
CD19+, %	17.62± 3.5	18.11± 3.09	0.443
CD4 +,%	43.56 ±7.39	46.64± 6.71	0.005
CD8 +,%	35.60 ± 6.28	37.70± 6.11	0.156
CD4 +/CD8 +	1.25 ± 0.16	1.25 ± 0.10	1.000
CD16-56 +, %	20.3 ± 4.30	21.97 ± 3.46	0.534
Activity	11.73 ± 1.89	12.38± 2.76	0.030
CD45 +, %	93.07 ± 1.20	93.28 ± 1.92	0.028

(p: 0.007). There was no statistically significant differences in the comparison of 16 neonates' lymphocyte counts and subgroup rates 48 hours after and before the period of phototherapy (p> 0.05) (Table 3).

DISCUSSION

Exposure to UVB triggers a multitude of molecular and cellular changes in skin. Although a great amount of ultraviolet (UV) light is absorbed in the epidermis, a small but important part of it passes through epidermis and reaches the dermis. In this way, T-lymphocytes located around the capillaries of the papillary dermis and T-lymphocytes in the postcapillary venules of the papillary dermis may be exposed to low dose UV light. Exposure of cells to visible light in vitro induced DNA strand break, sister chromatid exchange, and mutations were detected in experiments. In addition to causing DNA damage in the skin, UVB also modulates the immune system in distant lymphoid compartments.[1,2,5,8,9]

The immunosuppressive effects of solar radiation are mediated mostly by the middle wavelength range (UVB, 290-320 nm). Therefore, the vast majority of photoimmunologic studies utilized UVB. There is also evidence that the long wavelenght range (UVA, 320-400 nm) can affect the immune system although its effects are less pronounced.[10] In our study, long wavelenght range phototheraphy lamps were used.

One of the major hallmarks of UVB-induced immunosuppression is the fact that the immune system is affected in a rather specific than general fashion.[11] Many experimental models have shown that particular antigen-specific immune responses are suppressed by UVB radiation, while other immune reactions are not affected.[12] There have been a few studies dealing with the effects of UV on the immune system. In a study, UVB exposure inhibited the expansion of effector CD4+ and CD8+ T cells in skin-draining lymph nodes.[13] In a study on adults, a significant decrease in both B and T subgroups after exposure to UV light has been reported.[6] Neill et al. showed a decrease in CD16+/56+ subgroups after UVB exposure.[7] In a study during and after narrowband ultraviolet B (UVB) treatment, there were no differences in the number of circulating lymphocytes, lymphocyte subsets or cells expressing NK markers and controls. However significant decrease in the circulating CD4+ count during treatment of narrowband UVB was detected firstly in this study.[14] However, in another study it was shown that in patients who were exposed to acute UV light, increase in CD4+ cells in the skin two days after UV exposure was determined.[15]

It seems likely that phototheraphy could influence the immature immune system of the newborn, probably by direct effects on T-lymphocyte in the thin skin.[16,17] There are a limited number of studies evaluating the immune system after phototherapy in newborns. In a study evaluating the lymphoproliferative response and immunoglobu-

lins, phototherapy was shown to inhibit the lymphoproliferative response.[18] In a study from our country, lymphocyte subgroups were reported to be increased 72 hours after phototherapy, but at a non-significant rate, while TNF-α, IL-1β and IL-8 levels were reported to have increased significantly.[2] In our study, the CD4+ subgroups significantly increased eight hours after phototherapy ($p< 0.05$). Increased CD4+ subgroups in early period after phototheraphy may be associated with effects of phototheraphy on immune system in that time and/or using different wavelenght and dosing for UV radiation. Further studies are necessary to explore these factors.

In an in-vitro study, UVB was reported to kill most of the T-cells in a dose-dependent manner.[6] In a study on adults, low dose UVB light is suggested to cause a decrease in CD4+ and CD8+ cells after phototherapy, but at nonsignificant rates.[19] It was also reported that phototoxic effect manifested 48-72 hours after exposure to UVB, and not acutely.[6] In our study, there was no significant difference in lymphocyte subgroups 48 hours after phototherapy (Table 3).

UVB is also known induce the release of a variety of cytokines.[20,21] While the secretion of proinflammatory cytokines including interleukin (IL)-1, IL-6,IL-8 and TNF-α may play an important role in local and systemic sunburn reaction, the release of immunosuppressive cytokines such as IL-4 and IL-10 may contribute to its beneficial therapeutic effects.[22,23] Furthermore, UVB treatment suppresses the type axis as defined by IL-12, IFN-γ and IL-8, and can selectively reduce pro-inflammatory cytokine production by individual T cells.[11,24] In another study, it was reported that phototheraphy caused to increase IL-2, IL-10 production and to decrease IL-1β secretion.[3] Kurt et al. described that at 72 h of exposure to phototheraphy increased serum TNF-α, IL-1β and IL-8 levels significantly, while IL-6 level at the same time did not significantly change.[2] Blood cytokine levels couldn't measured in our study.

No adverse effects of bilirubin on lymphocyte subgroups have been reported in previous studies. In a study of Kurt et al., no significant difference was determined in lymphocyte subgroups and serum levels of IL-1β, IL-6, IL-8 and TNF-α between icteric babies before phototherapy and the control group.[2] However, Haga et al. showed that bilirubin inhibits the induction of cytotoxic T lymphocyte activity, and this defect may result from the impaired responsiveness against IL-2.[25] In our study, we also determined no significant effects of hyperbilirubinemia on lymphocyte counts and subgroups.

In conclusion, phototherapy which is used in the treatment of neonatal hyperbilirubinemia, causes an increase in only CD4+ cells in the early period; however, it has no significant effects on lymphocyte subgroups 48 hours after phototherapy. Consequently, the effects of phototherapy on the immune system are not directly associated with T-lymphocytes. Further studies are warranted to elucidate these possible effects of an increase in CD4+ cells in the early periods on the immune system.

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