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# A chromosomal-effect study of intensive phototherapy versus conventional phototherapy in newborns with jaundice☆

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ABSTRACT

In this study, we aimed to make a comparison between chromosomal effects caused by conventional phototherapy and intensive phototherapy in jaundiced newborns. The study group included 83 newborns with gestation age of ≥35 weeks, and on days 3–10 after birth. Newborns were divided into four groups on the basis of total serum bilirubin (TSB) levels upon admission and need for phototherapy. The intensive group ($n = 19$) consisted of newborns who received light-emitting diode (LED) phototherapy, the conventional group ($n = 23$) consisted of newborns who received conventional phototherapy, the jaundiced control group ($n = 21$) consisted of newborns whose TSB levels were higher than 10 mg/dL (average = 13.7 ± 1.5 mg/dL) on admission and who did not receive phototherapy, and the non-jaundiced control group ($n = 20$) consisted of newborns whose TSB levels were less than 5 mg/dl (average = 3.6 ± 0.8 mg/dL). TSB level of the intensive group at admission was 20.2 ± 1.3 mg/dL, whereas the level of conventional group was 19.6 ± 1.5 mg/dL. Blood samples were taken from all infants on admission to determine sister chromatid exchange ($SCE_1$) frequency. Blood sampling was repeated on discharge ($SCE_2$) of infants who had received phototherapy. Demographic information, hospitalization details and the rate of decline in TSB were recorded, and frequencies of $SCE_1$ and $SCE_2$ were compared. There was no difference in demographic information among the four groups. $SCE_1$ frequencies in 50 metaphases were evaluated in the intensive, conventional, jaundiced control and non-jaundiced control groups, and the $SCE_1$ frequency was determined as 9.37/cell, 9.54/cell, 9.23/cell and 6.17/cell, respectively. The $SCE_1$ frequency of the jaundiced groups (intensive, conventional and newborns-with-jaundice control group) was significantly higher than that in the non-jaundiced control group ($p = 0.001$). There was no significant difference between the intensive group and the conventional group in $SCE_2$ frequency (13.5/cell vs 13.55/cell, $p = 0.39$). $SCE_2$ frequency was higher than $SCE_1$ frequency in both the intensive and conventional groups ($p = 0.001$). A strong correlation was found between admission TSB and $SCE_1$ frequency ($p = 0.001$; $r = 0.79$). The rate of decline in TSB was higher in the intensive group compared with the conventional group (0.26 mg/(dL h) vs 0.14 mg/(dL h); $p = 0.001$). We found that intensive and conventional phototherapies similarly increase SCE frequency in newborns. There was a strong, positive correlation between the TSB-on-admission level and $SCE_1$ frequency. In the light of this study, we may conclude that intensive and conventional phototherapies may have an effect on chromosomes in jaundiced newborns. TSB levels higher than 10 mg/dL are, too, reported hazardous on chromosomes. Further studies are warranted to elucidate this relationship.


## 1. Introduction

Although phototherapy has been used for the treatment of neonatal jaundice for more than 50 years, the most efficacious phototherapy method with the least side effects has not been developed yet. The reported side effects of phototherapy have been subject to extensive and controversial debate, and include rashes, loose green stool, water loss, oxidative injury, and dehydration [1,2]. However, chromosomal side effects of the phototherapy began to be reported

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in some recent experimental studies [3,4]. DNA damage caused by phototherapy in jaundiced newborns has been, thereafter, shown in clinical studies [5,6].

Efficacy of phototherapy is dependent on the colour (wavelength) and intensity (irradiance) of the light emitted during phototherapy, the exposed body surface area, and the duration of exposure [2]. The American Academy of Pediatrics (AAP) defines intensive phototherapy as irradiance of at least 30 mW/(cm$^2$ nm) in the 430–490 nm band, and suggests intensive phototherapy to treat neonatal jaundice for gaining a faster bilirubin decrease [7]. Recently, high-intensity gallium nitride light-emitting diodes (LEDs) have been developed and studied as possible light sources for intensive phototherapy of neonatal jaundice [8]. Blue LEDs emit a high-intensity narrow band of blue light overlapping the peak spectrum of bilirubin breakdown [8], resulting in potentially shorter treatment times [9]. LEDs are also power-efficient, they are light in weight, produce less heat, and have a longer lifetime [8,10]. Although efficacy studies about the LEDs were performed, and the technique is being used much more than ever before in neonatology services, there is no study investigating whether there are chromosomal side effects in jaundiced newborns. There are studies concerning side effects of conventional phototherapy on either DNA or chromosomes.

Sister chromatid exchange (SCE) is a cytogenetic indicator arising during replication of damaged DNA templates from reciprocal DNA interchanges between sister chromatids during the replication process [11]. SCEs can arise from DNA damage occurring before DNA replication [12]. They can occur at certain rates normally, and as a consequence of exposure to ultraviolet light, ionizing radiation, environmental hazards, chemotherapeutics, viral infections, psoralens-plus-ultraviolet A (PUVA) therapy, and in malignancies [13].

We investigated in a clinical study using SCE analysis whether there are any chromosomal side effects of conventional and/or intensive phototherapy.

## 2. Materials and methods

This study was conducted between January 2008 and May 2008 on neonates with jaundice admitted to two neonatal units of different centers in Turkey. The study protocol was approved by the Ethics Committee of each Institute. Informed consent was obtained from all parents of the newborns involved in the study.

### 2.1. Patient selection

The study group included 83 newborns with gestation age of ≥35 weeks and on days 3–10 after birth. Patients were divided into four groups on the basis of the total bilirubin levels in serum (TSB) upon admission, and the need for phototherapy. The groups were formed as: an intensive group who needed phototherapy according to the suggestions of the AAP, and received intensive phototherapy ($n$ = 19). The conventional group received conventional phototherapy ($n$ = 23) [7]. We designed two different control groups in order both to make a comparison between the two therapy groups, and to investigate any chromosomal side effects of serum bilirubin independent of phototherapy. The infants with jaundice (TSB level ≥10 mg/dL) but excluded from phototherapy because the levels were below the AAP suggestions were placed in the jaundiced control group ($n$ = 21); the ones with no apparent jaundice (TSB level ≤5 mg/dL) were assigned to the non-jaundiced control group ($n$ = 20).

Newborns with a gestational age of <35 weeks or >42 weeks were defined as pre-term and post-term, respectively, and were not included in the study. Other exclusion criteria were a smaller or larger body weight than normal for gestational age, determined on the basis of the Colorado intrauterine growth charts [14], direct bilirubinemia, multiple gestation, any systemic disease, neonates that needed phototherapy within the first 3 days of life, any congenital malformation, respiratory distress, glucose-6-phosphate dehydrogenase deficiency, clinical- or culture-proven sepsis, and inability to initiate or maintain oral feedings within 3 h after birth due to various reasons.

### 2.2. Phototherapy

Phototherapy was applied to the intensive group with Neoblue® LED phototherapy system (Natus Medical Inc. San Carlos, CA, USA, intensity = 35 μW/(cm$^2$ nm), spectrum 450–470 nm). An Elektro-Mag® M304, Philips TL 20W/52 with a pair of blue-ray fluorescent lamps, intensity = 10 μW/(cm$^2$ nm) and spectrum 450–560 nm was used in the conventional group. Before starting phototherapy on a subject, the irradiance was checked with a photoradiometer (Fluoro-lite 451®, Minolta/Air Shields, USA). Our aim was to maintain the irradiance above 35 μW/(nm cm$^2$) during intensive phototherapy and above 10 μW/(nm cm$^2$) in the conventional group. The irradiance of the lamps was measured weekly, and if decreased, lamps were replaced whenever necessary, to maintain this irradiance. All infants were exposed, completely unclothed with their eyes and genitals covered, to continuous phototherapy that was interrupted only for feeding, cleaning and blood sampling. The infants' weights and temperatures were monitored. Gestational ages, types of feeding, ages at phototherapy, possible side effects concerning the phototherapy (weight loss, rashes, diarrhea, and thermal changes), TSB level at initiation of phototherapy and at termination of phototherapy, as well as duration of phototherapy of all the subjects were recorded.

Randomization in phototherapy groups was maintained according to admission to the hospital. The first patient was included in the conventional group, and the other ones were placed in either group alternately in order. The phototherapy was stopped, as recommended by the AAP, when the TSB decreased to a level of at least 2 mg/dL below the suggested AAP guideline, unique for each patient.

### 2.3. Blood samples

The first blood samples of the newborns involved in the study were taken for phenylketonuria screening, Guthrie's Test, within 3–10 days after birth when they entered the newborn outpatient clinic. Peripheral veins were used and a 0.5-mL blood sample for TSB analyses, and another 0.5 mL for pre-treatment SCE frequency ($SCE_1$) were collected. The samples for $SCE_1$ were transferred to the genetics lab within 1 h after collection in heparinized pediatric tubes. The routine serum tubes for TSB analyses were covered with aluminum foil to avoid exposure to sunlight, and immediately sent to the labs for analysis. TSB levels of subjects in phototherapy groups were checked every 12 h after hospitalization. The last sample in which the TSB had decreased to a level of 2 mg/dL below the suggested AAP guideline was conveyed at room temperature to the genetics lab within max 1 h in order to analyze the frequency of SCE ($SCE_2$). $SCE_2$ analysis was not carried out in the jaundiced and non-jaundiced control groups.

### 2.4. Analysis of sister chromatid exchange

For SCE analysis, 0.5 mL of heparinized blood was drawn from each individual. Cultures were established by adding 0.25 mL of blood to 5 mL karyotyping medium (Biological Industries, Beit Haemek, Israel) with 2% phytohaemagglutinin M (PHA) (Biological Industries, Beit Haemek, Israel), and incubating for 24 h at 37 °C. A 5-bromo-2′-deoxyuridine (BrdU) (Sigma, St. Louis, MO, USA) solution was added to a final concentration of 5 μg/mL. Lymphocytes were cultured in the dark for 48 h and metaphases were blocked during the last 2 h with Colcemid (Biological Industries, Beit Haemek, Israel) at a final concentration of 0.1 μg/mL. Further processing included hypotonic treatment, fixation, slide preparation and Fluorescein plus Giemsa (FPG) staining for the detection of SCE [15]. Fifty second-division metaphases were scored on coded slides by a single observer, and expressed as the number of SCEs/cell per subject. Staffs performing the SCE analysis were blinded to the study.

### 2.5. Statistics

SPPS 13.0 for Windows® (SPSS Inc., Chicago, IL) was used for statistics in the study. A $p$-value of <0.05 was considered as statistically significant.

The convenience to normal distribution in continuous variables was analyzed by Kolmogorov–Smirnov's Test of One Sample at the end of which the results were negative, and analysis was continued with non-parametric tests. Results are expressed as means in descriptive statistics with regard to the normal distributed variables. The Kruskal–Wallis and Mann–Whitney $U$-tests were used to determine significance. Pearson's Correlation Analysis was used for the assessment of relation between the parametric variables. A Wilcoxon's Rank Test was performed for the assessment of any difference between the first and second values of the dependent groups.

## 3. Results

Eighty-three newborns were involved in the study, 19 of whom were in the intensive group, 23 in the conventional, 21 in the jaundiced control group, and 20 in the non-jaundiced controls. The groups analyzed did not show statistically significant differences in terms of birth weight, age and weight at the moment of application, route of delivery, and type of feeding. TSB levels differed significantly as expected. No difference was seen between intensive and conventional phototherapy groups ($p$ = 0.4); the TSB levels of these groups were higher than those of the two control