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Assessment of DNA damage in babies treated with phototherapy for Neonatal jaundice.

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Abstract

Jaundice is one of the most common conditions which requires medical attention in neonates. In addition to the short-term side effects, phototherapy may result in DNA damage. In this study the extent of DNA damage was assessed before and after phototherapy in cases with neonatal jaundice by single cell gel electrophoresis (Comet Assay). Eighty babies with neonatal jaundice were chosen for the study. Among them 40 babies whose total serum bilirubin level was >15mg/dl and received phototherapy formed the case group and the other 40 babies with total serum bilirubin level <15mg/dl who did not require phototherapy formed the control group. The comet head diameter was decreased in post phototherapy group (42.37±10.3) compared to pre phototherapy study group (49.34±10.7) with p < 0.05. The tail length was increased in post phototherapy group (25.19±11.77) compared to pre phototherapy (7.95±14.25) study group and controls (2.28±0.8) with p value < 0.05. We conclude that phototherapy causes significant DNA damage in babies with neonatal jaundice.

Keywords: Neonatal jaundice, Phototherapy, Comet Assay, DNA damage.

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Introduction

Phototherapy is the most widely used form of therapy for newborn infants with hyperbilirubinemia in order to decrease the body burden of neurotoxic bilirubin. Phototherapy's noninvasive nature , easy availability, low cost and few side effects reported initially have almost led to the assumption that it is innocuous [1]). Phototherapy converts unconjugated bilirubin to its oxidation products as well as to photo and structural isomers, which are easily eliminated through gastrointestinal tract or in urine [2]. Phototherapy reduces bilirubin by using light energy to change the shape and structure of bilirubin, converting it to molecules that can be excreted even when normal conjugation is deficient. Absorption of light by dermal and subcutaneous layers, bilirubin induces a fraction of the pigment to undergo several photochemical reactions that occur at very different rates. The relative contributions of the various reactions to the overall elimination of bilirubin are unknown, although in vitro and in vivo studies suggest that photoisomerisation is more important than photodegradation. Bilirubin elimination depends on the rates of formation as well as the rates of clearance of the photoproducts. Photoisomerization occurs rapidly during phototherapy, and isomers appear in the blood long before the level of plasma bilirubin begins to decline. Phototherapy lights in current use do not emit significant erythemal uv radiation. In addition, the plastic cover of the lamp and in the case of preterm infants, the incubator filter out UV light [3]. The present study was conducted to evaluate DNA damage in babies who received phototherapy for neonatal jaundice using Comet assay.

Material and Methods

This case control study was conducted from September 2010 to May 2012. The study was approved by the Institute Scientific Advisory and Ethical Committee. Informed consent was obtained from the parents of the babies included in the study. Forty term babies with serum bilirubin level > 15 mg/dl who required phototherapy formed the cases for the current study. Forty term babies with clinical jaundice whose serum bilirubin level <15mg/dl who did not require phototherapy were the control group. Infants with congenital malformation, birth asphyxia, sepsis; and those with signs and symptoms suggestive of severe illness and babies who had received phototherapy before enrolement were excluded. Two ml of peripheral blood was collected from cases and controls in a heparinized tubes under sterile and aseptic conditions. The blood samples collected from the cases in prephototherapic phase as well as after forty eight hours of receiving phototherapy were utilized for comet assay.

Conventional phototherapy were given to all 40 cases at 420-470 nm wavelength with blue fluorescence lamp. The eyes were protected with eye shield and the external genitalia with diapers. The single cell gel electrophoresis (SCGE) assay or comet assay was done to assess DNA damage from peripheral lymphocytes [4]. The slides stained with silver nitrate are observed under a bright-field light microscope and captured using CCD camera. The captured images were analysed using commercially available software. The parameters such as Tail length, Head diameter, Percentage of DNA in head, percentage of DNA in tail were measured. Results were analysed by students paired & unpaired t- test using Instat Graphpad software and p value < 0.05 was taken as significant

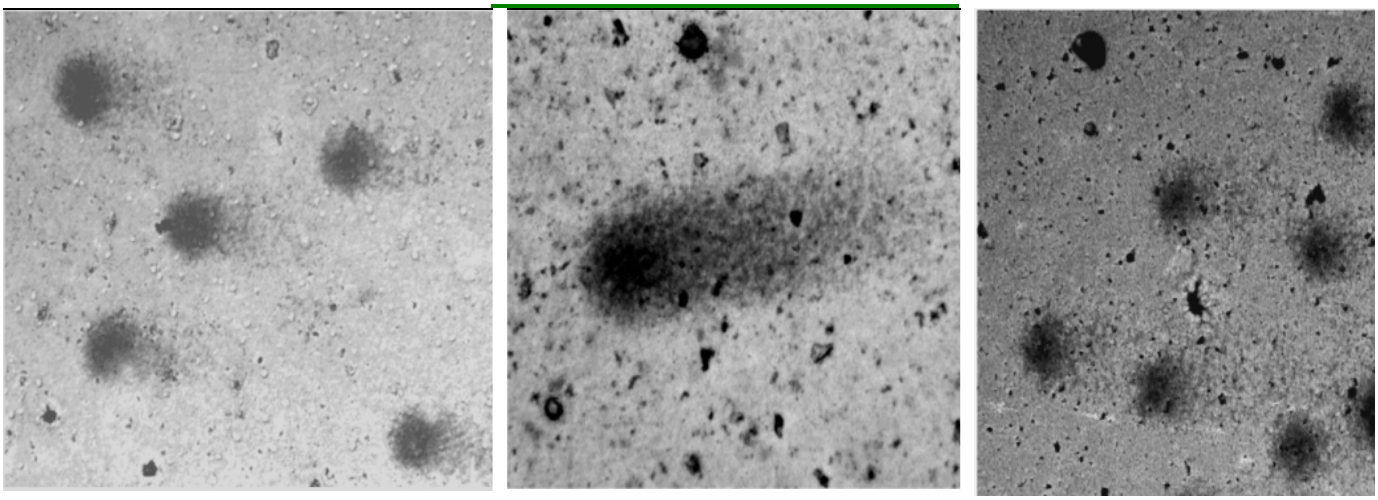
Results

The age and the birth weight of the babies were not significantly different among cases and controls. The mean comet head diameter in prephototherapy cases was 49.34µm and in post phototherapy 42.37µm and for controls 41.33µm. The head diameter is significantly decreased in post phototherapy compared to prephototherapy and controls. Similarly, the mean comet tail length of post phototherapy cases was 25.19µm and in pre phototherapy 7.95µm and for controls 2.28 µm. The tail length was significantly increased in post phototherapy compared to pre phototherapy and controls indicating DNA damage after phototherapy (Fig 1-3). The percentage of DNA in head and tail of the comet is inversely proportional to each other. The percentage of DNA in the tail was increased significantly in cases compared to controls .

Table 1. *Mean Comet parameters in cases and controls*

Comet parameters	**Comet parameters** (Mean±SD) **Cases** (n = 40)		**Controls** (n = 40) (C)	**P Value**
	Pre phototherapy (A)	**Post phototherapy(B)**		
Head diameter µm	49.34±10.7	42.37±10.3	41.33±8.4	A vs B * A vs C*
Tail length µm	7.95±14.25	25.19±11.77	2.28±0.8	A vs B * A vs C*
% DNA in head	92.8±1.9	81.13±11.76	93.85±2.48	A vs B * A vs C*
% DNA in tail	7.18±1.95	18.86±11.7	6.14±2.48	A vs B * A vs C*

**P< 0.05*



1: PRE PHOTOTHERAPY *2: POST PHOTOTHERAPY* *3: CONTROL*

Figures 1-3. *Comet appearance in babies with jaundice before and after phototherapy and in controls*

Discussion

Neonatal jaundice is one of the most common clinical conditions which requires medical attention. Bilirubin concentration reaches a peak within 3 to 5 days of birth among term babies and lasts for less than 2 weeks in physiological jaundice. The present study was undertaken to assess the phototherapy induced DNA damage in neonatal jaundice.

Tatli et al., have observed that DNA damage increased significantly with the duration of phototherapy as revealed by measurements at 24, 48, 72 hours [5]. In our study we measured DNA damage before starting and 48 hours after phototherapy. We observed significant increase in DNA damage after phototherapy and our findings are consistent with that of Tatli et al.

Endogenous mononuclear leukocyte DNA strand breaks is a well known type of DNA damage. Aycikek et al., investigated DNA damage before and after phototherapy in newborns with jaundice. They observed significant increase in DNA damage in both conventional phototherapy and intensive phototherapy (6). They collected blood samples before and after phototherapy in different babies which was the drawback of their study. In our study we collected blood samples from same babies before and after 48 hours of phototherapy to avoid inter individual variations.

Karadag et al, found that intensive and conventional phototherapies increased sister chromatid exchange (SCE) frequency [7]) . Study by El Abdin et al., showed that DNA damage in circulating lymphocytes has significantly increased before and after phototherapy compared to controls in circulating lymphocytes [8].

Karakukcu et al, reported that comet scores and plasma catalase activites in hyperbilirubimenic newborns were significantly higher before phototherapy compared with the values after giving phototherapy and also in the control group. According to him high serum bilirubin level has genotoxic effects and phototherapy does not cause an increase in DNA oxidation or induce the genotoxic effects of bilirubin [9]. ut in our study we found more DNA damage after phototherapy although total serum bilirubin among them has decreased.

This study shows that there is increased DNA damage in the jaundiced neonates and higher in those who received phototherapy as a treatment for jaundice. Even though phototherapy is the mainstay of treatment for neonatal jaundice, phototherapy induces DNA damage which is in addition to the damage caused by the serum bilirubin. So usage of phototherapy should be restricted to those with significant hyperbilirubinemia.

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