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Role of ultraviolet rays filters and antioxidants in prevention of DNA damage and oxidative stress associated with phototherapy in jaundiced term neonates

Ahmed Saad Mady Saad (1), Gamila Solyman Mohamed Elsaeed (2).

1-M.Sc of pediatrics, specialist of pediatrics, Tala General Hospital (Fever Department), Monufia Governorate, Ministry of Health and Population, Egypt.

2-MD medical biochemistry, professor of medical biochemistry, National Research Centre, Cairo, Egypt.

Abstract

Background: phototherapy induces DNA (deoxyribonucleic acid) damage by direct and indirect (oxidative) effects which are prevented by ultraviolet (UV) filters and antioxidant.

Objective: our goal was to assess DNA damage and oxidative stress associated with phototherapy and to prove the efficacy of UV filters and antioxidants for prevention of that DNA damage.

Methods: The study included 160 jaundiced neonates who had been exposed to phototherapy for at least 48 hours divided into four groups, 40 neonates per each group, the first (control) group received phototherapy only, the second group received antioxidants before and during phototherapy, the third group received phototherapy under umbrella of UV filters and the fourth group received phototherapy under both UV filters and antioxidants. DNA damage was assayed by (comet assay). Plasma total antioxidant capacity (TAC) and total oxidant status (TOS)

levels were also measured then oxidative stress index (OSI) was calculated for the all four groups before and 48 hours after phototherapy.

Results: the first group showed significant DNA damage accompanied with severe deterioration in all oxidative stress parameters by about 19%, the second group showed decreasing as regard DNA damage and oxidative stress parameters deterioration to about 9%, the third group showed more decreasing than group II to about 5%, but on the other hand the fourth group showed complete DNA protection from damage with no changes in oxidative stress parameters.

Conclusion: phototherapy cause DNA damage that can be completely prevented by combined concurrent use of UV filters and antioxidants.

Keywords: UV rays filters, Melatonin, Antioxidants, Neonatal Hyperbilirubinemia, Phototherapy, DNA damage, Oxidative stress, Comet Assay, Ionizing Radiation, Free Radicals.

Introduction

Phototherapy is used for neonates with hyperbilirubinemia to decrease the neurotoxic bilirubin [1]. Untoward effects on DNA have been demonstrated in vitro [2]. Fluorescent lamps and light emission diode LED lamps are used in phototherapy. A fluorescent lamp produces UV light that then causes a phosphor coating on the inside of its bulb to glow visible light [3] Not all the UV striking the phosphor gets converted into visible light [4] .

Ionizing radiation carries enough energy to free electrons from atoms thereby ionizing them. Higher UV spectrum are ionizing [5] .

To some extent, visible light and also ultraviolet A (UVA) have been proven to result in formation of reactive oxygen species in skin which cause indirect DNA damage [6] .

Free radical damage to DNA can occur as a result of exposure to ionizing radiation [7] .

Antioxidant molecules work as scavengers, this way reducing reactive oxygen and nitrogen species bioavailability [8] .

Melatonin is a powerful antioxidant and believed to be the most effective lipophilic antioxidant [9] . So, this study was conducted to assess the importance of using both antioxidants and UV filters either together or separately for complete prevention or significant decreasing of DNA damage and oxidative stress induced by UV rays and visible light associated with phototherapy in jaundiced term neonates.

Patients and methods

The authors testify that the Ethical Committee of Research in National Research Centre, Cairo, Egypt approved our research and a written consent had been signed by all parents of all neonates shared in this study.

Patients

A randomized controlled study was conducted in neonatal intensive care unit at Tala general hospital, Menufia governorate, Egypt in cooperation with medical biochemistry department at national research center, Cairo, Egypt during the period from June 2017 to February 2019, and included 160 term (37-40 weeks) jaundiced neonates aged from 1 to 10 days old were in need for phototherapy. The patients were divided into four groups.

Group I: Forty term jaundiced neonates received phototherapy only. Group II: Forty terms jaundiced neonates received phototherapy with oral antioxidants therapy one hour before and during phototherapy as melatonin 10 mg/kg/4 hours for 48 hours [10] , vitamin A 30,000 IU single dose only (3.3 IU = 1 μg retinol) [11] , vitamin E 400 mg/day for two days (1.5 IU = 1 mg alpha-tocopherol) [12] and selenium 45 mcg/day for two days [13] .

Group III: Forty term jaundiced neonates received phototherapy under umbrella of fully transparent UV rays filters (sheets for LED lamps or sleeves for fluorescent lamps).

Group IV: Forty term jaundiced neonates received phototherapy under umbrella of fully transparent UV rays filters in addition to combined concurrent administration of melatonin and other antioxidants (as the same doses in group II) before and during phototherapy.

All the four groups had been investigated before and 48 hours after phototherapy for DNA damage by single cell gel alkaline electrophoresis (comet assay) and oxidation status by (TOS) and (TAC) then (OSI) has been calculated according to the following formula:

OSI= [(TOS)/ (TAC)/100].

Exclusion criteria:

Neonates with perinatal asphyxia, pre-term or post-term neonates, neonates with congenital malformations, infants of diabetic mothers, proved or suspected neonatal sepsis, those neonates whose bilirubin levels dropped to normal before completing 48 hours of exposure to phototherapy, those with symptoms and signs suggestive of serious illnesses, neonates with direct hyperbilirubinemia, and neonates in the zone of exchange transfusion.

Methods

Three ml of peripheral blood was collected from the all patients before phototherapy as well as after 48 hours of phototherapy. Blood samples were withdrawn from peripheral vein puncture under strict sterile and aseptic precaution into two EDTA tubes, approximately 1.5 ml of blood per each EDTA tube. The first tube was stored at 4°C in the dark to prevent further DNA damage and was processed for (comet assay) within 2 hours to assess DNA damage in peripheral lymphocytes. The slides are stained with silver nitrate are observed under a bright field light

microscope and captured using charged-coupled device CCD camera. The parameters such as Tail length, Head diameter, Percentage of DNA in head, percentage of DNA in tail were measured. Results were analyzed by students paired & unpaired t- test using Instat Graphpad software and p value < 0.05 was taken as significant. The second tube was centrifuged to obtain the plasma then stored at -80°C until further analysis for TAC and TOS levels which were measured by Erel's methods. [14,15] the percentage of TOS level to TAC level was regarded as the OSI [16,17] To perform the calculation, the result unit of TAC, mmol Trolox equivalents per liter, was changed to μmol Trolox equivalent per liter, and the OSI value was calculated as follows:

OSI = [(TOS, μmol per liter/(TAC, μmol Trolox equivalent per liter)/100].

Statistical methods

Continuous data was presented in the form of mean and standard deviation. Comparing between data before and after the treatment within each group was performed using paired t test. Comparing between the data in the four groups was performed using one way analysis of variance (ANOVA). P was considered significant if it is less than 0.05.

Results

Regarding the demographic and clinical data of the studied neonates and their mothers, no significant differences in age (hours), gestational age (weeks), weight (gram), bilirubin level (mg/dl), sex, mode of delivery, and DNA damage scores regarding bilirubin level in the pre and post-phototherapic results between the neonates in the all groups.

In group I, there was a significant DNA damage accompanied with severe deterioration in all oxidative stress parameters after 48 hours of phototherapy by about 19% in general as shown in table 1.

Table (1). Comparison of comet assay parameters and oxidative stress parameters between pre-phototherapy results and 48 hours post-phototherapy results in group I.

Variables	Group I		
	Pre-phototherapy (Mean ± SD)	Post-phototherapy (Mean ± SD)	p- value
Head diameter µm	49.973 ± 6.334	40.641 ± 8.837	<0.00001
Tail length µm	8.395 ± 8.155	26.07 ± 6.899	<0.00001
% DNA in head	92.673 ± 4.034	80.233 ± 7.823	<0.00001
% DNA in tail	7.283 ± 4.041	19.703 ± 3.701	<0.00001
TOS (µmol H_2O_2 equiv /L)	13.435 ± 3.133	18.58 ± 2.388	<0.00001
TAC (mmol Trolox equiv /L)	0.907 ± 4.096	0.643 ± 2.074	<0.00001
OSI (arbitrary unit**)**	1.481 ± 1.218	2.935 ± 1.389	<0.00001

In group II (who received antioxidants), DNA damage as well as oxidative stress parameters were significantly reduced after 48 hours phototherapy to about 9% in general in comparison to group I as shown in table 2.

Table (2). Comparison of comet assay parameters and oxidative stress parameters between pre-phototherapy results and 48 hours post-phototherapy results in group II.

Variables	Group II		
	Pre-phototherapy (Mean ± SD)	Post-phototherapy (Mean ± SD)	p- value
Head diameter µm	50.275 ± 8.115	45.428 ± 4.514	<0.00001
Tail length µm	7.955 ± 7.942	16.731 ± 5.419	<0.00001
% DNA in head	92.988 ± 4.003	84.642 ± 8.527	<0.00001
% DNA in tail	7.013 ± 5.003	15.358 ± 4.523	<0.00001
TOS (µmol H_2O_2 equiv /L)	13.245 ± 4.943	16.113 ± 3.386	<0.00001
TAC (mmol Trolox equiv /L)	0.912 ± 5.101	0.731 ± 4.081	<0.00001
OSI (arbitrary unit**)**	1.475 ± 1.179	2.204 ± 1.237	<0.00001

In group III (who received phototherapy under UV filters), DNA damage was more markedly reduced and oxidative stress parameters showed more markedly reduced deterioration after 48 hours phototherapy

to about 5% in general in comparison to groups I and II as shown in table 3.

Table (3). Comparison of comet assay parameters and oxidative stress parameters between pre-phototherapy results and 48 hours post-phototherapy results in group III.

Variables	Group III		
	Pre-phototherapy (Mean ± SD)	Post-phototherapy (Mean ± SD)	p- value
Head diameter	49.538 ± 7.458	47.121 ± 8.457	<0.00001
Tail length	8.164 ± 8.247	12.557 ± 3.548	<0.00001
% DNA in head	92.241 ±5.034	87.527 ± 6.658	<0.00001
% DNA in tail	7.759 ± 4.041	12.473 ± 7.754	<0.00001
TOS (μmol H_2O_2 equiv /L)	13.752 ± 5.133	15.154 ± 3.658	<0.00001
TAC (mmol Trolox equiv /L)	0.924 ± 4.096	0.839 ± 3.145	<0.00001
OSI (arbitrary unit**)**	1.488 ± 1.168	1.806 ± 1.258	<0.00001

Group IV (who received phototherapy under combined concurrent UV filters and antioxidants), showed complete DNA protection from damage with no change in oxidative stress parameters as shown in table 4.

Table (4). Comparison of comet assay parameters and oxidative stress parameters between pre-phototherapy results and 48 hours post-phototherapy results in group IV.

Variables	Group IV		
	Pre-phototherapy	Post-phototherapy	p- value
Head diameter	50.025 ± 3.574	50.034 ± 5.576	0.448
Tail length	8.415 ± 3.245	8.417 ± 7.247	0.129
% DNA in head	92.795 ±6.058	92.819 ±6.061	0.171
% DNA in tail	7.205 ± 8.124	7.181 ± 4.129	0.240
TOS (μmol H_2O_2 equiv /L)	13.468 ± 4.325	13.465 ± 3.374	0.417
TAC (mmol Trolox equiv /L)	0.911 ± 5.086	0.916 ± 4.069	0.830
OSI (arbitrary unit**)**	1.478 ± 1.258	1.471 ± 1.194	0.577

Table (5). General comparison of comet assay parameters and oxidative stress parameters between overall collective mean of pre-phototherapy results in all four groups and separate means of post-phototherapy results in each group.

Variables	Pre-phototherapy	Post-phototherapy			
	All groups	Group I	Group II	Group III	Group IV
Head diameter µm (mean)	49.953	40.641	45.428	47.121	50.034
Tail length µm (mean)	8.232	26.07	16.731	12.557	8.417
% DNA in head (mean)	92.674	80.233	84.642	87.527	92.819
% DNA in tail (mean)	7.315	19.703	15.358	12.473	7.181
TOS (µmol H_2O_2 equiv/L) (mean)	13.475	18.58	16.113	15.154	13.465
TAC (mmol Trolox equiv/L) (mean)	0.914	0.643	0.731	0.839	0.916
OSI (arbitrary unit**)** (mean)	1.481	2.935	2.204	1.806	1.471

Discussion

In this study we found significant DNA damage after phototherapy in group I and our findings are consistent with that of other investigators [18 -21]. Furthermore, Tatli *et al.* [22] have observed that DNA damage increased significantly with the duration of phototherapy as revealed by measurements at 24, 48, 72 hours. In our study we measured DNA damage before starting and 48 hours after phototherapy.

Karadag *et al.* [23] and Aycicek *et al*. [18] found that intensive phototherapy induced more DNA damage than that induced by conventional phototherapy

In this study we found significant deterioration in all oxidative stress parameters after phototherapy in group I as regard significant increase in TOS and OSI accompanied with significant decrease in TAC and our findings are consistent with that of Aycicek *et al.* [18] and Aycicek and Erel., [24] except with TAC levels which were not altered significantly by phototherapy in their studies but Bohles *et al.* [25] reported a significant decrease in TAC during phototherapy just like us.

As regards group II in our study we have given high tolerable doses of multiple strong antioxidants especially melatonin to the neonates of group II before and during phototherapy and our post-phototherapy results showed markedly reduced DNA damage and also markedly reduced deterioration in oxidative stress parameters after 48 hours phototherapy to about 9% in general in comparison to group I which probably because antioxidants prevented oxidative effects of both UV rays (indirect) and visible light but they didn't prevent direct effect of UV rays then in group III we have used UV filters only and our post-phototherapy results showed more improving than group II as regard decreasing DNA damage and oxidative stress parameters deterioration to about 5% in general which probably because UV filters prevented direct and indirect (oxidative) effect of UV rays but they didn't prevent oxidative effect of visible light but on the other hand, in group IV we have used combined concurrent antioxidants (as the same as in group II) and UV filters (as the same as group III) and our post-phototherapy results were marvelous as they showed complete DNA protection from damage with no change in oxidative stress parameters which probably because this combination prevented completely both UV rays (direct and indirect) effects in addition to visible light oxidative effect. Until the date of writing this study we didn't find any published study about using antioxidants or UV filters either separately or together to prevent or to decrease DNA damage and oxidative stress associated with phototherapy in jaundiced term neonates, so we can't compare our results in group II, III, IV with anybody else.

We think more studies are needed to evaluate our work and also we hope to be the pioneers of using both UV filters and antioxidants especially melatonin either together for complete prevention or separately for significant decrease of DNA damage and oxidative stress associated with phototherapy in jaundiced term neonates.

Conclusion:

Phototherapy cause significant DNA damage. We recommend the combined concurrent use of UV rays filters and antioxidants before and during phototherapy.

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