

EFFECTS OF PHOTOTHERAPY ON SERUM PARAOXONASE ACTIVITY AND TOTAL ANTIOXIDANT CAPACITY IN NEWBORN JAUNDICE

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ABSTRACT

Objective: Phototherapy is an efficient and commonly used form of therapy for the treatment of neonatal hyperbilirubinemia. Paraoxonase 1 (PON1) is an antioxidative enzyme, which eliminates lipid peroxides. The aim of our study was to investigate the effect of phototherapy on serum PON1 activity and total antioxidant capacity (TAC) in hyperbilirubinemic full-term newborns.

Material and Method: The study was performed on 40 full-term newborns between 3 to 15 days of age exposed to phototherapy. Serum PON1 activity and TAC levels of the babies were determined before and after phototherapy by spectrophotometric assays.

Results: We have found that PON1 activity was not significantly affected by phototherapy whereas TAC levels were decreased significantly after phototherapy ($p<0.001$).

Conclusion: Our findings demonstrated that phototherapy has no direct effect on PON1 activity. Also, decreased TAC levels might have resulted from increased oxidative stress which may lead to consumption of antioxidant molecules.

Keywords: Phototherapy, neonatal hyperbilirubinemia, paraoxonase, total antioxidant capacity *Nobel Med 2014; 10(3): 48-51*

FOTOTERAPİNİN YENİDOĞAN SARILIĞINDA SERUM PARAOKSONAZ AKTİVİTESİ VE TOTAL ANTIOKSİDAN KAPASİTEYE ETKİLERİ

ÖZET

Amaç: Fototerapi neonatal hiperbilirubineminin tedavisinde günümüzde oldukça yaygın ve etkili olarak kullanılan bir tedavi şeklidir. Paraoksonaz 1 (PON1) lipid peroksitleri elimine eden bir antioksidan enzimdir. Çalışmamızın amacı fototerapinin miadında doğan yenidoğanlarda serum PON1 aktivitesi ve total antioksidan kapasite (TAC)'ye etkisini araştırmaktır.

Materyal ve Metod: Çalışma, miadında doğan ve 3-15 günlük olup fototerapi tedavisi alan 40 yenidoğan üzerinde gerçekleştirildi. Bebeklerin serum PON1

aktivitesi ve TAC seviyeleri fototerapi öncesi ve fototerapi sonrası spektrofotometrik olarak ölçüldü.

Bulgular: Sonuçlarımız PON1 aktivitesinin fototerapiden anlamlı bir şekilde etkilenmediğini fakat TAC seviyelerinin fototerapi sonrasında anlamlı şekilde azaldığını gösterdi ($p<0,001$).

Sonuç: Bulgularımız fototerapinin PON1 aktivitesine direkt etkisi olmadığını göstermiştir. TAC seviyelerindeki azalma ise antioksidan moleküllerin tüketimine neden olan artmış oksidatif stresten kaynaklanıyor olabilir.

Anahtar Kelimeler: Fototerapi, yenidoğan hiperbilirubinemi, paraoksonaz, total antioksidan kapasite *Nobel Med 2014; 10(3): 48-51*

INTRODUCTION

About 60% of normal newborns become clinically jaundiced during the first week of life.¹ Since high serum bilirubin concentrations can injure the newborn's central nervous system it must be treated immediately.¹⁻³ High serum bilirubin concentrations are treated with either exchange transfusion or phototherapy which is an efficient and commonly used form of therapy.^{1,2,4} Phototherapy converts bilirubin into more polar water-soluble isomers that can be readily eliminated without hepatic conjugation for excretion.^{1,2} Phototherapy is considered to be a noninvasive and safe type of therapy with few side effects.² However, several recent studies have reported that this might not be true. It has been reported that phototherapy is a photodynamic stress and can induce oxidative stress, lipid peroxidation and peroxidative damage of tissues.^{5-10,11}

Paraoxonase 1 (PON1), a protein of 354 amino acids, is a major determinant of the antioxidant system and the antiinflammatory function of the lipoprotein. PON1 is almost exclusively associated with high density lipoprotein cholesterol (HDL-C). Thus, its antioxidant activity is largely attributed to PON1 located on it. Indeed, PON1 protects both HDL-C and low density lipoprotein cholesterol (LDL-C) from oxidation. The beneficial effects of PON1 has been attributed to its ability to reduce lipid hydroperoxides. PON1 was also shown to hydrolyze atherogenic products of oxidative lipid modification such as phospholipid peroxides and cholesterol ester hydroperoxides. PON1 also could play a role in the release of more active form of antioxidant/antiinflammatory molecules. There is growing evidence which show PON1's protective role in atherosclerosis.^{12,13}

Although phototherapy was generally found to increase oxidative stress in previous studies, there is no study concerning the effect of phototherapy on PON1 activity.³⁻⁷ Therefore, we have evaluated the effect of phototherapy on PON1 activity and total antioxidant capacity (TAC) levels, a simple indicator of the activity of all antioxidants, in hyperbilirubinemic newborn infants.

MATERIAL and METHOD

Patients

The subjects included in this study were babies born at Meram Faculty of Medicine, Selçuk University and admitted to neonatal unit. Forty full-term hyperbilirubinemic newborn infants (19 M/21 F) between 3 to 15 days of age and 37 completed weeks

(estimated according to the last menstrual period and/or the New Ballard score assessment) were investigated. The infants were being breastfed and had no known risk factor for hyperbilirubinaemia. Serum total bilirubin levels of all infants were above 18 mg/dl. Infants with severe congenital malformation, birth asphyxia, sepsis, respiratory distress, maternal diabetes or hemolytic-type hyperbilirubinaemia symptoms suggestive of serious illness were excluded from the study. The study was approved by the ethical committee of Meram Faculty of Medicine, Selçuk University, Konya, Turkey and informed consent was obtained from the parents of the infants.

The naked newborn, except for those who wore diapers and eye patches, was placed inside an incubator with a phototherapy system consisting of 16 white fluorescent tubes (Philips TL 20W/52) and 40 cm above. The light energy of the phototherapy unit, measured by a standard photometer, was 22-26 mW/cm²/nm. All babies were exposed to continuous phototherapy, except while being fed and cleaned. No side effects of phototherapy were observed during the procedure. Blood samples were collected before and after phototherapy and total bilirubin, TAC levels and PON1 activity of the sera were determined. The blood samples were kept at room temperature for 30 min. for the completion of the coagulation. Then sera were separated from the cells by centrifugation at 3000 rpm for 10 min. Total bilirubin was measured using commercial kits (Beckman Coulter, Fullerton, CA, USA) immediately and serum samples were stored at -80°C until the day of analysis of the other parameters.

Measurement of paraoxonase activity

Paraoxonase activity was measured using paraoxon as substrate. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm at 37°C.¹⁴ PON1 activity was expressed as U/L serum.

Measurement of the total antioxidant capacity

Total oxidant status of sera was determined by a commercially available kit based on automated measurement method (Rel Assay Diagnostic, Gaziantep, Turkey). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar H₂O₂ equivalent per liter (µmol H₂O₂ equiv./L). →

Table 1: The demographic characteristics and baseline biochemical parameters of infants

Number of newborns	40
Males/Females	19/21
Age (days)	5.90 ± 2.52
Weight (kg)	3.0 ± 0.4
Duration of phototherapy (h)	29.7 ± 8.6
Gestational age (week)	38.42 ± 1.34

Table 2: Comparison of serum parameters before and after phototherapy

Parameters	Before phototherapy	After phototherapy	P
Paraoxonase (U/L)	25.29 ± 21.0	21.93 ± 19.69	0.073
TAC (mmol Trolox equiv./L)	5.80 ± 0.95	4.26 ± 0.57	<0.001
Total serum bilirubin (mg/dl)	21.84 ± 3.6	11.29 ± 1.3	<0.001

TAC: Total antioxidant capacity

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Version 13.0). All data were expressed as mean ± standard deviation (SD). Statistical differences between the groups were evaluated using an independent samples t-test. The correlations between variables were performed by Pearson's Correlation test. Differences were considered significant at a probability level of $p < 0.05$.

RESULTS

The demographic characteristics and baseline biochemical parameters of infants are presented in Table 1. Mean and SD values of PON1 activity and TAC levels before and after phototherapy are presented in Table 2. As seen from the Table 2, PON1 activity was decreased slightly after phototherapy but the difference was not statistically significant. However, TAC levels were decreased significantly after phototherapy ($p < 0.001$). Also, we have found a significant positive correlation between TAC and bilirubin levels before ($r = 0.461$, $p < 0.01$) and after ($r = 0.370$, $p < 0.05$) phototherapy.

DISCUSSION

Our results show that phototherapy has no significant effect on serum PON1 activity whereas TAC levels decreased significantly after phototherapy. This finding is in accordance with the findings of Shekeeb Shahab et al.⁵ who found that TAC levels decreased significantly after phototherapy in hyperbilirubinemic infants.

Two mechanisms may be responsible for the decreased TAC levels after phototherapy in hyperbilirubinemic

infants. One mechanism might be increased oxidative stress which may have resulted in consumption of antioxidant molecules, because many recent studies reported that phototherapy has a negative impact on numerous parts of the oxidant/antioxidant system. Gathwala et al. have shown that thiobarbituric acid reactive substances (TBARS) were significantly higher in icteric preterm newborns after phototherapy.⁶ In another study, Shekeeb Shahab et al. have reported that phototherapy had a significant contribution to oxidative stress as indicated by lipid peroxidation measured as malondialdehyde (MDA) levels in hyperbilirubinemic infants.⁵ They also observed a significant decrease in glutathione (GSH) and TAC levels after phototherapy. Likewise, in our a previous study we have found that MDA levels were increased significantly after phototherapy in hyperbilirubinemic infants.⁷ Also, Ayçicek et al. have found that vitamin C and uric acid levels were significantly lower and total oxidant status (TOS), lipid hydroperoxide and oxidative stress index levels were significantly higher after phototherapy than the values before phototherapy.⁸ TAC levels were slightly but not significantly decreased after phototherapy. Demirel et al. have found that TOS levels were significantly increased and TAC levels were slightly but not significantly decreased after phototherapy in hyperbilirubinemic infants.⁹

We believe that another source of significantly decreased TAC levels might be decreased levels of bilirubin which is also an antioxidant.¹⁵ Recent studies have shown that the antioxidant effects of mildly elevated serum bilirubin levels, as well as activation of heme oxygenase, may protect against diseases associated with oxidative stress.¹⁶ In a study conducted by Stocker et al. bilirubin and biliverdin have been shown to inhibit the formation of linoleic acid hydroperoxides significantly.¹⁵ Also, Belanger et al. studied the antioxidant capacity of bilirubin in infants and showed that plasma antioxidant capacities of jaundiced infants were related to the level of bilirubin as they showed a decrease in antioxidant capacity after exchange transfusion, although the antioxidant capacity of the donor blood was similar to infant plasma antioxidant capacity after transfusion.¹⁷

Our finding of significant positive correlation between TAC and bilirubin both levels before and after phototherapy suggests that bilirubin functions as a natural antioxidant. Also, Dogan et al. have found a positive correlation between serum total bilirubin and TAC levels in hyperbilirubinemic newborns.¹⁸

In our study, PON1 activity was slightly but not significantly decreased after phototherapy. This finding shows that phototherapy has no direct effect on →

PON1 activity. In the literature we have found no other study measuring PON1 activity in hyperbilirubinemic newborns after phototherapy. Therefore this is the first study measuring PON1 activity in these subjects.

It has been reported that PON1 enzyme activity was sensitive to oxidative stress and readily inactivated by oxidants.^{13,19,20} However, PON1 activities of our subjects did not change significantly after phototherapy in spite of increased oxidative stress as proved by decreased TAC. Thus, there may be an adaptation of PON1 activity to increased oxidative

stress in our subjects. Therefore, further investigations are needed to evaluate the effect of phototherapy on the activity of PON1 enzyme.

In conclusion, our findings suggest that phototherapy has no serious effect on PON1 activity. Also, significantly reduced TAC levels of infants exposed to phototherapy may suggest the need of supplementation of antioxidants to them.

* The authors declare that there are no conflicts of interest.



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