

*Research Article***The Effect of Phototherapy on Expression of Bax and Bcl2 Genes in Neonates with Jaundice**

Mohamed F. Afifi*, **Abdel Hakeem A. Abdel Hakeem***,
Emad A. Abd El naeem** and **Marwa I. Abd Elrazic***

* Department of Pediatrics, El-Minia Faculty of Medicine, El Minia, Egypt.

** Department of Clinical pathology, El-Minia Faculty of Medicine, El Minia, Egypt.

Abstract

Objectives: to evaluate the effect of phototherapy on expression of BAX and Bcl2 genes in full term with hyperbilirubinemia. It comprised 18 full term and 18 preterm neonates with indirect hyperbilirubinemia who received phototherapy for 24 h, and 9 apparently healthy full term and 9 healthy preterm neonates with normal serum bilirubin level, as a control group. **Determination:** of the anti-apoptotic effect(s) of BCL2 gene and the pro-apoptotic effect(s) of (Bax) gene was achieved by quantitative assay of its product, (BCL2) protein, and BAX protein by ELISA. **Results:** there were significant decrease in the serum levels of bcl2 after phototherapy ($P = 0.001$) and significant increase in serum levels of Bax protein after phototherapy ($P < 0.001$) in hyperbilirubinemic full term neonates. **Conclusion:** Hyperbilirubinemia does not influence on apoptosis, whereas phototherapy does and induces apoptosis in peripheral blood of hyperbilirubinemic full-term infants.

Keywords: hyperbilirubinemic ,phototherapy,apoptosis.

Introduction

Very high levels of unconjugated bilirubin are neurotoxic. Phototherapy is a simple and effective way to reduce the bilirubin level. Complications from phototherapy are rare and generally mild. Most term babies have 'physiological' jaundice which responds to a short period of phototherapy, and requires no other treatment ⁽¹⁾.

Phototherapy may lead to oxidative injury to the cell membrane and, as a result, increases the levels of lipid peroxidation products. Free oxygen radicals in excess may give rise to injury to host cells and may induce DNA strand breaks ⁽²⁾. Recently phototherapy may cause DNA damage and induces apoptosis in peripheral blood lymphocytes of full-term infants ⁽³⁾.

Apoptosis is a form of genetically programmed cell death, which is both evolutionally conserved and tightly regulated at a molecular level⁽⁴⁾. Two pathways mediate Apoptosis– extrinsic and intrinsic. The extrinsic pathway is mediated

by death receptors, such as Fas and the tumor necrosis factor receptors. The

intrinsic pathway is mediated by the conserved family of Bcl-2 proteins, which are important for regulating apoptosis through mitochondria⁽⁵⁾.

Cells are generally equipped with DNA repair mechanisms to decrease the levels of DNA damage. However, neonates have been found to have lower DNA repair and antioxidant capacities compared with adults⁽⁶⁾.

The BCL2 family of proteins constitutes a critical control point in apoptosis residing immediately upstream of irreversible cellular damage, where family members control the release of apoptogenic factors from the mitochondria. The cardinal member of this family, BCL2, was originally discovered as the defining oncogene in follicular lymphoma. There are two genes (BCL2 and P53) that control the process of apoptosis. BCL2 is an oncogene

which blocks apoptosis. It can be called cell death suppressor gene because of its direct regulation of apoptosis. A high concentration of BCL2 protein protects the cell from apoptosis⁽⁷⁾.

Patients and methods

Patients

This is a prospective observational study was conducted on 36 neonates with indirect hyperbilirubinemia (18 full term and 18 preterm) admitted to the neonatal intensive care unit (NICU) Minia university children hospital, and had received intensive phototherapy based on AAP guidelines, this study also included 18 apparently healthy neonates (9 full term and 9 preterm) as a control group in the 1st two weeks of life.. Neonates with birth asphyxia, sepsis, infants of diabetic mothers, and neonates with congenital anomalies and neonates with cholestatic jaundice all were excluded from the study.

All patients and healthy controls were subjected to full perinatal history and thorough clinical examination. Complete Blood Count, reticulocyte count, liver enzymes and serum albumin level, total and direct bilirubin, RH and blood group to mothers and babies, all were done to enrolled neonates.

Serum Bcl2 and serum BAX protein levels were assayed by ELISA technique. One sample was taken at the admission and another one after 24 hour of intensive phototherapy exposure (Bilisphere 360).

Sampling

under complete aseptc technique before phototherapy five millilitre of peripheral venous blood was withdrawn: 1 ml in EDTA vacutainer for C.B.C., Reticlocyte count, 2 ml in plain vacutainer for Serum bilirubin level (total and direct bilirubin), RH and blood group, Liver enzymes and serum albumin level and 2 ml in EDTA

vacutainer for, serum BCL2 level and BAX protein level.

After phototherapy 4 millilitre of peripheral blood was withdrawn; 2 millilitre in EDTA vacutainer for: serum BCL2 and BAX protein and 2 millilitre in plain vacutainer for serum bilirubin level.

Phototherapy

The type of phototherapy used in the study is Intensive phototherapy systems which consisted of 12 white fluorescent tubes (Philips TL03, Ontario,Canada) placed within 20 cm under and above the infant's front and back. The infants were placed naked, except for a diaper and eye patches, in an incubator or intensive phototherapy unit (Bilicrystal, Medes-time,or Bilisphere 360, Marcinelle, Belgium). The light energy of the phototherapy units was 30–34 μ W cm⁻² nm⁻¹ in the 430–490- nm band. Phototherapy was continuously applied to jaundiced neonates except during feeding and care.

Result

There were no differences between the groups in terms of postnatal age, birth weight, and sex. Also laboratory data no differences between the groups in terms of CBC, reticulocyte count, liver enzymes and serum albumin levels. Comparison between hyperbilirubinemic full term, control, and preterm, control regarding serum bilirubin level, serum levels of BCL2 and BAX Protein, there were highly significant higher level of total and direct bilirubin ($p < 0.001$) before and after phototherapy in hyperbilirubinemic neonates, but there were no significant difference in serum levels of bcl2 and Bax protein before phototherapy ($P > 0.05$) but highly significant decrease in the serum levels of bcl2 after phototherapy and highly significant increase in serum levels of Bax protein after phototherapy Figure (1, 2,3 and4). There were a significant positive correlation between serum levels of Bcl2 and total bilirubin after phototherapy, and significant negative correlation between Bax and total bilirubin after phototherapy (Tabel 1 and 2).

Table (1): Correlation between total bilirubin and serum levels of BCL2 and BAX Protein, in hyperbilirubinemic full term neonates before and after phototherapy

Group		T. bilirubin pre		T. bilirubin post	
		R	P value	R	P value
	BCL2	0.178	0.480	0.702	0.001*
Cases Full term	BAX	-0.222	0.375	-0.846	<0.001*

Table (2): Correlation between total bilirubin and serum levels of BCL2 and BAX Protein, in hyperbilirubinemic preterm neonates.

Group		T. bilirubin pre		T. bilirubin post	
		R	P value	R	P value
	BCL2	0.153	0.543	0.624	0.006*
Cases Preterm	BAX	0.049	0.847	-0.768	<0.001*

Figure (1): comparison of serum levels of Bcl2 protein in hyperbilirubinemic neonates and control in full term neonates before and after phototherapy.

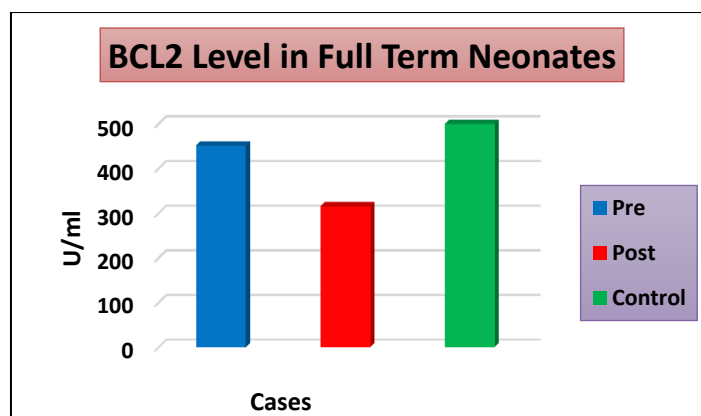


Figure (2): comparison of serum levels of Bcl2 protein in hyperbilirubinemic neonates and control in preterm neonates before and after phototherapy.

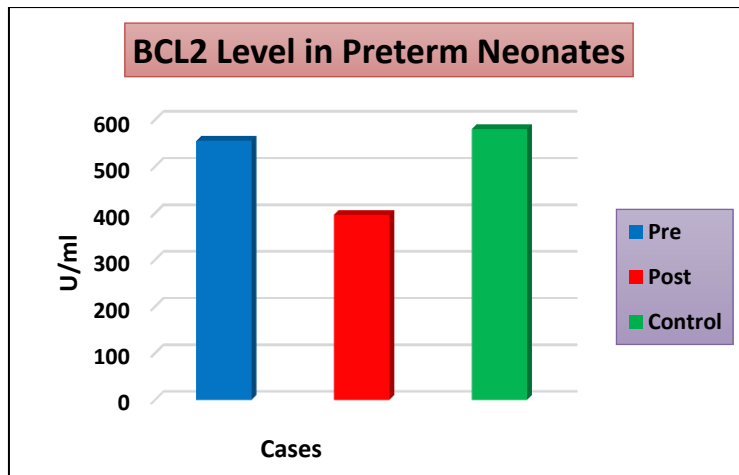


Figure (3): comparison of serum levels of Bax protein in hyperbilirubinemic neonates and control in full term neonates before and after phototherapy.

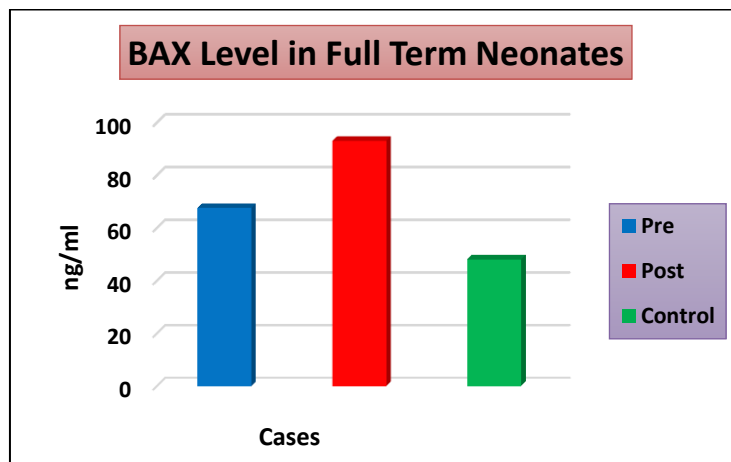
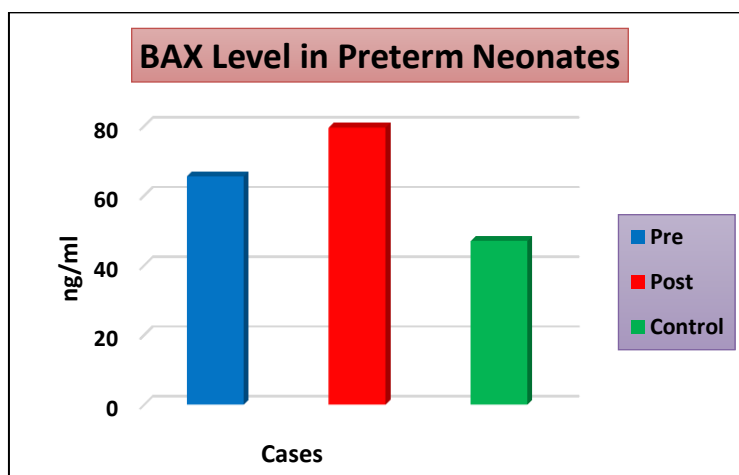


Figure (4): comparison of serum levels of Bax protein in hyperbilirubinemic neonates and control in preterm neonates before and after phototherapy.



Discussion

In the present study concerning the clinical and laboratory data, we found that there

was no significant difference between hyperbilirubinemic full term neonates and control regarding age, sex, weight, CBC, liver function and albumin. The non-significant differences between cases and control group is important to ensure the homogenization of the studied groups to get accurate results as much as possible when comparing between groups and to refer any differences to the studied factors. Also we observed that there was significant increase in reticulocyte count in hyperbilirubinemic full term neonates that is an evidence of hemolysis. In agreement of our study, El-Abdin et al.,⁽⁸⁾ Who found that reticulocyte count showed high statistical significant differences in the cases before phototherapy compared to controls, Xavier et al.,⁽⁹⁾ found that babies with reticulocytosis had earlier onset of jaundice, though the peak bilirubin and duration of phototherapy were similar to that of neonates without reticulocytosis.

In the current study, we observed that there were highly significant increases in serum level of total and direct bilirubin in hyperbilirubinemic full term neonates before phototherapy compared to controls. After phototherapy it became significantly lower compared to its level before phototherapy this show the efficacy of intensive phototherapy in aggrement with our finding El-Abdin M.Y.Z et al.,⁽⁸⁾ also Abdelazeem KS et al⁽¹⁰⁾ who showed that the use of intensive phototherapy in the management of pathological hyperbilirubinemia is very effective in lowering total serum Bilirubin when its level is within 2-3 mg/dl (34-50 μ mol/l) of the exchange transfusion level and in reducing the use of the invasive maneuver of exchange transfusion with its serious neurological complications. It has also succeeded in reducing the duration of phototherapy and subsequently the duration of hospital stay and economic burden. Also we found that before phototherapy serum levels of bcl2 and Bax protein were not statistically significant ($P > 0.05$) between hyperbilirubinemic full term neonates and controls but after phototherapy, there were highly significant decrease in the serum levels of bcl2 ($P = 0.001$) and highly significant

increase in serum levels of Bax protein so hyperbilirubinemia did not influence apoptosis in peripheral blood of hyperbilirubinemic full-term neonates. Also there were non significant correlation between total bilirubin, bcl2 and bax protein before phototherapy.

Our study disagreed with El-Abdin et al.,⁽⁸⁾ who found that the serum level of BCL2 Protein was lower among the cases before phototherapy compared to controls explaining that bilirubin and phototherapy had genotoxic effects, also Basu S, et al⁽¹¹⁾ reported DNA damage even with low bilirubin concentrations, suggesting possible genotoxic effects of bilirubin in unconjugated hyperbilirubinemia. On the other hand Mesbah-Namin et al.,⁽¹²⁾ found that the number of apoptotic cells before the incubation showed no significant differences between all three groups.

Furthermore, there was no correlation between hyperbilirubinemia and the number of apoptotic cells, although the study was done on apoptotic cells, the conclusions are similar to ours and could support the results of the present study. Yahia et al.,⁽³⁾ found no significant difference in P53 level (marker of apoptosis) in the tested groups before exposure to phototherapy; it has been shown that hyperbilirubinemia lacks the ability to induce any genotoxic effects on DNA in jaundiced neonates as they attributed this incidence to the fact that bilirubin has an important physiological antioxidant role, leading to its cytoprotective feature mediated by its sacrificial oxidation, also (Ramy et al.,⁽¹³⁾ reported that bilirubin did not influence DNA damage in the peripheral blood mononuclear cells of full-term infants.

In the present study we found that after phototherapy the serum levels of bcl2 were significantly lower compared to their levels before and serum levels of bax proteins were significantly higher compared to their levels before also significant positive correlation between Bcl2 and bilirubin and significant negative correlation between BAX and bilirubin in hyperbilirubinemic full term neonates.

In consistence with our finding El-Abdin et al.,⁽⁸⁾ who found that After phototherapy the serum levels of bcl2 became more significantly lower compared to its level before phototherapy and BAX messenger RNA expression was more significantly higher explaining that Phototherapy was found to induce more DNA damage than that was induced by high bilirubin level. Phototherapy seems to induce an evident down regulation of BCL2 level in newborn with hyperbilirubinemia as BCL2 and upregulation of BAX gene expression in neonates with hyperbilirubinemia, However, this does not guarantee that all damaged cells will undergo apoptosis. Yahia et al.,⁽³⁾ found that P53 levels significantly increased after exposure to phototherapy and there were significant positive correlations between the duration of phototherapy and markers of DNA damage.

In contrast, Mesbah-Namin et al.,⁽¹²⁾ found that no increased frequency of apoptotic cells was observed immediately after phototherapy (Ap0) in the phototherapy group compared to that in controls and explained that by different phases of apoptosis process and duration, The P53 level as well as plasma Bcl-2 and BAX genes expression are considered to be signals affecting apoptotic responses, and any changes in their levels are associated with apoptosis. Mohamed WW, and Niazy WH⁽¹⁴⁾, found that Phototherapy may induce genotoxic effect in peripheral blood lymphocytes of term infants with hyperbilirubinemia .

As before phototherapy, there were no significant differences between the four groups in terms of DNA damage, SCE frequency, TAC, or TOS ($p > 0.05$). After phototherapy, DNA damage, SCE frequency, and TOS were significantly higher in the intensive than in the conventional group ($p < 0.05$). In the phototherapy-treated groups, DNA damage and SCE frequency correlated positively with TOS ($p < 0.05$), Interesting study Ramy et al.,⁽¹³⁾ demonstrated that hyperbilirubinemia is not associated with DNA strand breaks, which

are a well-known type of DNA damage, both conventional and intensive phototherapy treatments increased DNA damage. The duration, but not the intensity, of phototherapy correlates with the degree of DNA damage.

Our study is the first to assess the effect of extensive phototherapy on apoptotic markers in hyperbilirubinemic preterm neonates, to our knowledge; we do not have data from other studies to compare.

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