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The Effects of Phototherapy on Magnesium, Vitamin D, Albumin, Calcium Levels, and Platelet Counts in Neonates With Hyperbilirubinemia

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Abstract

Introduction: Phototherapy is the standard treatment for neonatal hyperbilirubinemia, however, its effects on biochemical and hematological parameters remain incompletely understood. This study aimed to evaluate the impact of phototherapy on serum magnesium, vitamin D, albumin, calcium levels, and platelet counts in neonates with hyperbilirubinemia.

Methods: A prospective before–after study was conducted on 100 neonates receiving phototherapy. Blood samples were collected before treatment and 24 hours after phototherapy. Biochemical parameters were measured using standardized assays. Albumin gene expression and protein levels were assessed using real-time PCR and Western blotting. Paired t-tests were applied to compare pre- and post-treatment values. Pearson correlation and ROC curve analyses were performed to evaluate relationships and predictive performance.

Results: Phototherapy resulted in significant reductions in platelet counts ($P=0.031$), albumin levels ($P=0.028$), magnesium ($P=0.004$), vitamin D ($P=0.003$), and calcium levels ($P=0.006$). Magnesium demonstrated the highest diagnostic performance (AUC = 0.805), followed by the magnesium/albumin ratio (AUC = 0.766). A strong positive correlation was observed between magnesium and platelet counts ($r=0.736$, $P<0.01$). Albumin mRNA expression and protein levels significantly decreased following phototherapy ($P<0.05$).

Conclusion: Phototherapy induces significant biochemical and hematological alterations in neonates, particularly affecting magnesium, albumin, calcium, vitamin D, and platelet levels. Routine monitoring of these parameters during phototherapy is recommended to prevent potential complications.

Keywords: Neonatal hyperbilirubinemia, Phototherapy, Magnesium, Platelet count, Vitamin D, Albumin, Calcium

Introduction

Neonatal hyperbilirubinemia is a frequent clinical condition affecting approximately 60% of term and 80% of preterm neonates during the first week of life. It results primarily from immature hepatic bilirubin conjugation and increased hemolysis, leading to elevated serum bilirubin levels and visible jaundice. Although typically benign, severe hyperbilirubinemia may progress to kernicterus and permanent neurological damage if not appropriately managed. Phototherapy remains the cornerstone of treatment for neonatal jaundice. By converting unconjugated bilirubin into water-soluble photoisomers, phototherapy facilitates bilirubin excretion through bile and urine. Despite its established efficacy, growing evidence suggests that phototherapy may influence multiple physiological and biochemical processes in neonates.[1] Although phototherapy is effective in managing this condition, several studies have raised concerns regarding its potential impacts on various biochemical parameters in neonates, including electrolytes, hematological indices, and nutritional status.[2]

Several studies have reported electrolyte disturbances following phototherapy, particularly reductions in serum calcium and magnesium levels, which may result from altered melatonin secretion and increased renal excretion. Hypocalcemia has been associated with neuromuscular

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irritability and seizures, while hypomagnesemia may impair enzymatic activity and cardiovascular stability. For instance, shahriarpanah et al. (2019) [3] found that exposure to phototherapy could lead to a decrease in magnesium levels, which is critical for numerous physiological functions, including neuromuscular transmission and enzyme activity. Similarly, calcium levels, which are vital for bone mineralization and neuromuscular function, may be compromised. Furthermore, phototherapy has been associated with changes in platelet counts, which are crucial for hemostasis. A study by Wong et al. (2022)[4] observed that neonates undergoing phototherapy experienced a statistically significant decline in platelet counts post-treatment. This drop raises concerns regarding the potential increased risk of bleeding complications, warranting careful monitoring.[5]

Several studies have demonstrated that phototherapy may induce electrolyte imbalances, particularly hypocalcemia and hypomagnesemia, due to hormonal alterations and increased urinary excretion.[6,7] Previous research has also reported transient thrombocytopenia following phototherapy, potentially due to platelet activation and consumption.[8,9]

Vitamin D, essential for calcium homeostasis and skeletal development, may also be altered during phototherapy due to photodegradation or metabolic shifts. Disruptions in vitamin D status during early life may have long-term consequences on bone health.[10,11] Reduced serum albumin levels due to phototherapy could influence drug binding and efficacy in neonates, as albumin levels are critical for the pharmacokinetics of many therapeutic agents.[12] Given these potential effects, a comprehensive evaluation of biochemical and hematological alterations associated with phototherapy is essential. This study aimed to assess changes in serum magnesium, vitamin D, albumin, calcium levels, and platelet counts in neonates undergoing phototherapy for hyperbilirubinemia, as well as to investigate albumin gene expression and protein levels. We hypothesized that phototherapy would significantly modify these parameters, highlighting the need for close biochemical monitoring in neonatal care.

Materials and Methods

Study design

This observational study was conducted on 100 infants with hyperbilirubinemia who needed phototherapy as part of their treatment regimen (Ayatollah Kashani Hospital-Tehran, Iran) in a neonatal intensive care unit (NICU) for six months. The study protocol was approved by the Institutional Review Board, and informed consent was obtained from the parents or guardians of all enrolled infants. All infants received continuous phototherapy for 24 hours a day, and nurses monitored them for adverse reactions such as rashes or temperature instability. Blood samples were collected before the start of treatment (baseline/or control group) and 24 hours after the completion of phototherapy (subject group). Eligible participants included infants born at ≥34 weeks' gestation with a total serum bilirubin (TSB) level above the phototherapy threshold according to the American Academy of Pediatrics. Exclusion criteria included infants with underlying metabolic disorders, infants with diagnosed infections, or those who received any additional treatment (such as blood transfusion) during the study period.

Sample Size Calculation

Sample size was determined using power analysis (G*Power version 3.1). Assuming a medium effect size of 0.5 for paired comparisons, with a statistical power of 80% and an alpha level of 0.05, a minimum of 86 neonates were required. To compensate for potential attrition, 100 neonates were included.

Phototherapy Device

All neonates received continuous phototherapy using a blue LED phototherapy unit (Jiangsu Folee, China). Light intensity was maintained according to manufacturer recommendations and monitored throughout the treatment. Phototherapy was administered for 24 hours.

Blood Collection Kits

Sterile blood collection tubes (EDTA tubes for complete blood count and serum separator tubes for biochemical analysis) were used. The collection was performed using a standard venipuncture technique.

Magnesium Level Measurement

Serum magnesium levels were assessed using a colorimetric assay kit (Biorex fars-iran) from a reputable laboratory supplier. The assay was performed according to the manufacturer's instructions.

Platelet Count

A complete blood count (CBC) was performed using an automated hematology analyzer (SYSMEX-K21), which implements impedance and optical methods for accurate platelet counting.

Vitamin D, Bilirubin, Calcium, and Albumin Measurement

Vitamin D serum levels were processed using a chemiluminescent enzyme immunoassay kit (Pishtaz-Iran) and an ELISA reader. In addition, calcium, bilirubin and albumin levels were measured using a kit (Byrox Fars-Iran) on an automatic biochemical analyzer (Hitachi-Japan). Serum magnesium and calcium were measured using standard colorimetric assays as previously described.[13] Albumin measurement was performed according to established biochemical protocols.[14]

RNA Extraction and cDNA Synthesis

Total RNA was extracted from serum samples using an RNA extraction kit (Sinaclon, Iran), following the manufacturer's instructions. cDNA was synthesized from 1 μg of RNA using a reverse transcription kit (Pars Tous kit, Iran).

Primer Design

AlleleID® v6.0 software (Premier Biosoft Corporation, USA) was used to design gene-specific primers, and online primer blast software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to confirm their specificity to corresponding genes. Specific primers for human albumin (ALB) and a housekeeping gene, β-actin, were designed:

ALB Forward: 5'-GAA CTT CCT GGC CTC AAC CTA-3'
ALB Reverse: 5'-CCT GTT GCT GTC AGT CTC TTG-3'
β-actin Forward: 5'-ACAGAGCCTCGCCTTTGC-3'
β-actin Reverse: 5'-ATCACGCCCTGGTGCCT-3'

Quantitative PCR

An automated PCR amplification system (Roche Diagnostics, Switzerland) was used for the PCR amplification. Subsequently, the gene expression levels were normalized by comparison to the expression of β-actin using the $2^{-\Delta\Delta Ct}$ (fold change) formula. In addition, 2% agarose gel electrophoresis was used to confirm the size of PCR products when compared with a 10kb DNA ladder marker.

Western Blot Analysis

Serum proteins were separated using SDS-PAGE and transferred onto nitrocellulose membranes. After blocking, membranes were incubated with anti-albumin primary antibodies followed by HRP-conjugated secondary antibodies. Protein bands were visualized using enhanced chemiluminescence and quantified by ImageJ software.

Statistical Analysis

Data were analyzed using SPSS version 25. Normality was assessed using the Kolmogorov–Smirnov test. Paired t-tests were used to compare biochemical and hematological parameters before and after phototherapy. Pearson's correlation analysis was applied to assess relationships among variables. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of biomarkers. P-values < 0.05 were considered statistically significant.

Results

Characteristics of the patients

A total of 100 neonates (68 males and 32 females) were included. Mean gestational age was 37.0 ± 1.5 weeks, and mean birth weight was 2.5 ± 0.4 kg. The mean age at initiation of phototherapy was 10.05 ± 0.34 days. Mean serum bilirubin levels significantly decreased from 16.0 ± 0.6 mg/dL before phototherapy to 11.0 ± 0.8 mg/dL after treatment (P < 0.001).

Alteration of serum biochemical parameters in pre- and post-phototherapy groups

Paired t-tests were used to compare biochemical parameters before and after phototherapy. Infants with jaundice after phototherapy had significantly lower platelet counts and albumin levels compared to the control group before phototherapy (P < 0.05). Also, serum levels of magnesium, bilirubin, vitamin D, and calcium were significantly lower in post-phototherapy patients than in control subjects (P < 0.01). The patients after phototherapy also had a significant ratio of magnesium/vitamin D and magnesium/albumin (P < 0.01), and this ratio was higher in the group after phototherapy, and the largest changes after phototherapy in the measured values were related to platelets. (Table 1)

Table 1. Average levels of magnesium, vitamin D, calcium, bilirubin, albumin, and platelet counts before and after phototherapy for the studied neonates

Evaluation of sensitivities and characteristics of variables as predictive markers for phototherapy effects

ROC curve analysis was performed to evaluate diagnostic performance. Areas under the curve (AUCs) and P-values were obtained as follows: AUC = 0.805 and P < 0.05 for Magnesium; AUC = 0.766 and P < 0.05 for the Magnesium/Albumin ratio; AUC = 0.759 and P < 0.05 for Vitamin D; AUC = 0.744 and P < 0.05 for Calcium; AUC = 0.689 and P < 0.05 for PLT count; AUC = 0.678 and P < 0.05 for Albumin; and AUC = 0.627 and P < 0.05 for the Magnesium/Vitamin D ratio (Figure 1). The highest level of sensitivity and specificity was related to magnesium and the magnesium/albumin ratio.

Assessment of correlations among the studied parameters

Pearson's correlation coefficient study revealed many significant correlations among variables, as it is shown in Table 2. As described in Table 2, R values over 0.6 were considered strong correlations. Among all studied correlations, Magnesium with Platelet (r = 0.736, P < 0.01),

Table 1. Comparison of laboratory parameters before and after phototherapy in neonates with hyperbilirubinemia

Parameter	Pre-Phototherapy Mean ± SD	Post-Phototherapy Mean ± SD	P-value
Magnesium (mg/dL)	2.1 ± 0.5	1.8 ± 0.4	0.004
Bilirubin (mg/dL)	16 ± 0.6	11 ± 0.8	0.005
Platelet Count (× 10^3/mm^3)	250 ± 60	230 ± 50	0.031
Vitamin D (ng/mL)	25 ± 5	18 ± 4	0.003
Albumin (g/dL)	3.5 ± 0.3	3.0 ± 0.4	0.028
Calcium (mg/dL)	9.4 ± 0.5	8.9 ± 0.5	0.006
Magnesium/ Vitamin D ratio	0.084 ± 0.4	0.1 ± 0.04	0.003
Magnesium/ Albumin ratio	0.86 ± 0.3	1.12 ± 0.5	0.007

the Magnesium/Albumin ratio with Calcium (r = 0.674, $P < 0.01$), Platelet with Vitamin D (r = 0.601, $P < 0.01$), and Calcium with the Magnesium/Vitamin D ratio (r = 0.521, $P < 0.01$) were the strongest, respectively.

Real-Time PCR and Western Blot Results

Ct values showed a significant decrease after phototherapy, indicating a decrease in albumin expression in infants ($P < 0.05$). Western blot analysis showed distinct bands corresponding to albumin at about 66 kDa. The intensity of the bands decreased significantly after phototherapy, reflecting the lower albumin concentration ($P < 0.05$) (Figure 2).

Discussion

The present study demonstrated that phototherapy induces significant biochemical and hematological alterations in neonates with hyperbilirubinemia. Notably, serum magnesium, calcium, vitamin D, albumin levels, and platelet counts were all significantly reduced following phototherapy. These findings support growing evidence that although phototherapy is an effective treatment for neonatal jaundice, it is not metabolically inert and may influence multiple physiological systems. The observed reduction in serum magnesium levels is consistent with previous investigations reporting hypomagnesemia following phototherapy. Shahriarpanah et al[3] reported a significant decline in magnesium concentrations in neonates exposed to phototherapy, attributing this effect to increased renal excretion and hormonal changes.[15,16] Magnesium plays a crucial role in neuromuscular transmission and cardiovascular stability, and its deficiency may predispose neonates to irritability, arrhythmias, and seizures.[17,18] The strong correlation

Table 2. Correlation analyses were performed using the change values of biochemical parameters

		Magnesium	Bilirubin	Age	Platelet	Vitamin D	Albumin	Calcium	Magnesium/ Vitamin D ratio	Magnesium/ Vitamin D ratio	Magnesium/ Albumin ratio
Magnesium	R	1	.431**	.014	.736**	.448**	-.018	.336**	.336**	.325**	-.195
Bilirubin	R	.401**	1	-.040	.393**	.319**	.277**	.445**	.445**	.187	-.355**
Age	R	.014	-.040	1	.153	.004	.096	-.229*	-.229*	-.107	.069
Platelet	R	.446**	.393**	.153	1	.601**	.242*	.416**	.416**	.291**	-.262*
Vitamin D	R	.458**	.319**	.004	.246**	1	-.072	.316**	.316**	.152	-.260*
Albumin	R	-.018	.277**	.096	.242*	-.072	1	.066	.066	.130	-.141
Calcium	R	.326**	.445**	-.229*	.416**	.316**	.066	1	0.521**	.474**	-.413**
Magnesium/ Vitamin D ratio	R	.326**	.445**	-.229*	.416**	.316**	.066	0.301**	1	.474**	-.423**
Magnesium/ Albumin ratio	R	.325**	.187	-.107	.291**	.152	.130	.674**	.474**	1	-.054

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)
R values below 0.2 were interpreted as very weak correlations. R values between 0.2 and 0.4 were interpreted as weakly correlated, those between 0.4 and 0.6 as moderately correlated, those between 0.6 and 0.8 as strongly correlated, and those between 0.8 and 1.0 as very strongly correlated. Three strong correlations were found among the studied parameters: Magnesium with Platelet, Magnesium/Albumin ratio with Calcium

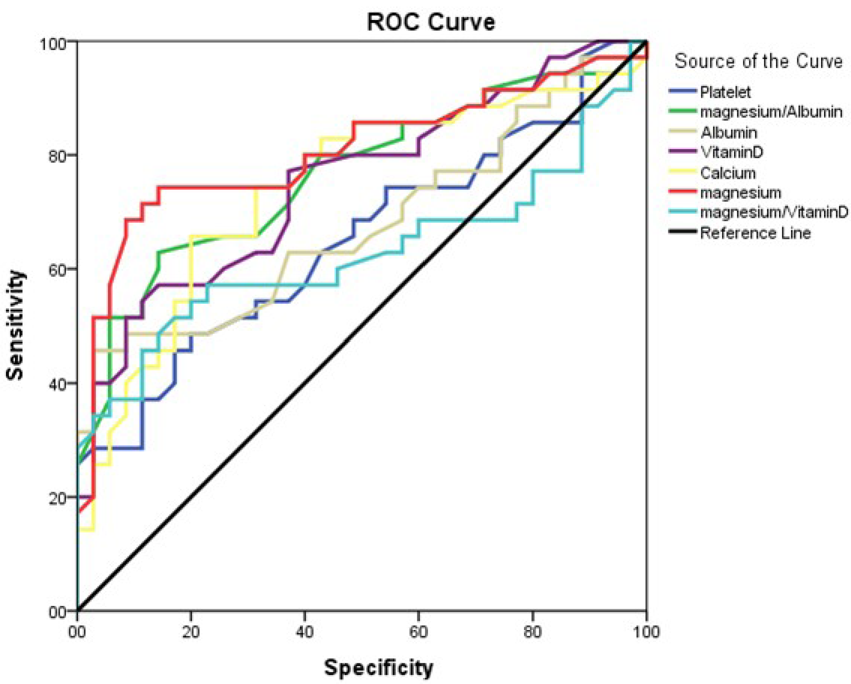

Figure 1. The ROC analysis of the studied parameters. The results proposed Magnesium and the Magnesium/Albumin ratio as the best biomarkers

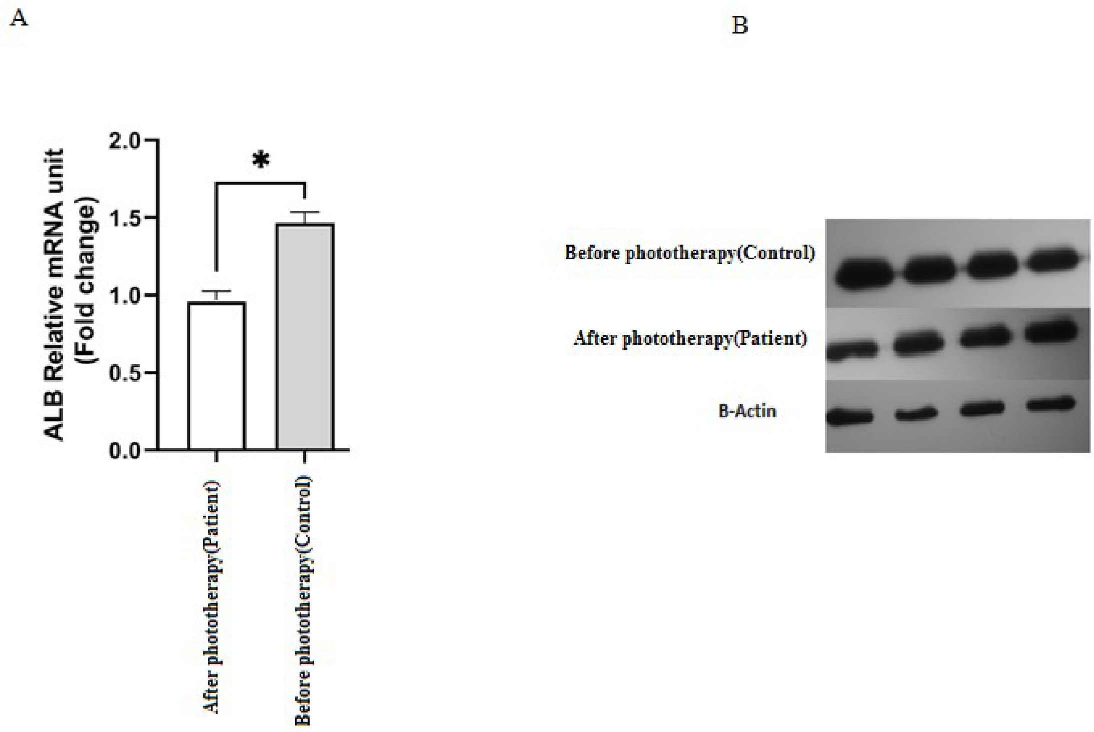

Figure 2. (A) mRNA expression of ALB gene, (B) ALB protein level measured by the densitometric method using ImageJ software

observed between magnesium levels and platelet counts in our study further suggests a potential interaction between electrolyte balance and hematological function. Similarly, our findings regarding calcium reduction align with earlier studies demonstrating phototherapy-induced hypocalcemia. Decreased melatonin secretion caused by light exposure may inhibit parathyroid hormone release, resulting in reduced calcium mobilization.[19] Hypocalcemia in neonates may manifest as neuromuscular excitability and convulsions, emphasizing the clinical importance of calcium monitoring during phototherapy.[20] The significant decline in platelet counts observed in our cohort corroborates previous reports of transient thrombocytopenia associated with phototherapy [21](Khera S et al.). Previous studies have documented similar reductions in platelet count, suggesting possible platelet activation and increased peripheral consumption during phototherapy. Although the observed decreases were generally mild, even moderate thrombocytopenia in neonates may increase bleeding risk, particularly in critically ill infants. Therefore, close monitoring of platelet counts is recommended throughout phototherapy, especially in high-risk neonates with pre-existing hematological disorders or low birth weight..[22] Vitamin D levels were markedly reduced following phototherapy, which contrasts with some studies reporting minimal changes but aligns with others suggesting photodegradation of vitamin D metabolites under light exposure. Given the essential role of vitamin D in bone mineralization and immune function, prolonged phototherapy may predispose neonates to suboptimal vitamin D status, potentially affecting long-term skeletal health.[23] The decrease in serum albumin levels, confirmed at both gene expression and protein levels, is of particular clinical relevance. Albumin serves as the primary binding protein for bilirubin and numerous medications.[24] Reduced albumin availability may influence bilirubin transport and drug pharmacokinetics, increasing the risk of toxicity. The consistency between molecular and biochemical findings strengthens the reliability of our results and highlights a novel mechanistic aspect of phototherapy-induced changes. ROC analysis identified magnesium and the magnesium/albumin ratio as the most sensitive indicators of phototherapy-related biochemical alterations. These markers may serve as early warning indicators for metabolic disturbances, allowing clinicians to intervene before clinical symptoms emerge. Collectively, these findings underscore the necessity of comprehensive biochemical monitoring in neonates undergoing phototherapy. Routine assessment of electrolytes, vitamin D, albumin, and platelet counts may enable early detection of adverse effects and improve neonatal outcomes.

Conclusion

In conclusion, our study highlights significant biochemical changes in infants undergoing phototherapy for hyperbilirubinemia. The observed decrease in serum levels of magnesium, platelet counts, vitamin D, calcium and gene expression, and albumin protein levels emphasizes the need for close monitoring in this vulnerable population. The findings suggest that although phototherapy is an effective treatment for the management of neonatal jaundice, it is not without potential complications that can affect the overall health of babies. Decreased magnesium and calcium levels may pose risks to neuromuscular function and bone health, respectively, while decreased platelet counts raise concerns about bleeding complications. Our study contributes to the growing body of evidence on the biochemical consequences of phototherapy and emphasizes the importance of a multidisciplinary approach to neonatal care.

Study Limitations

This study was limited by its single-center design and relatively short follow-up period. Long-term clinical consequences of the observed biochemical changes were not assessed. Future multicenter studies with extended follow-up are warranted to determine the long-term impact of phototherapy on neonatal metabolic health.

Authors' Contribution

Conceptualization: Rajab Mardani
Data Curation: Zahra Razaghi
Formal Analysis: Rajab Mardani
Investigation: Mohammad Javad Hossein Tehrani
Methodology: Zahra Razaghi
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Software: Mohammad Javad Hossein Tehrani
Supervision: Nayebali Ahmadi
Validation: Rajab Mardani
Writing–Original Draft: Rajab Mardani
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Competing Interests

None declared.

Ethical Approval

The biochemical parameters of this study were approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SUBMO.LASER.REC.1403.030). All experiments performed in this study were based on the Declaration of Helsinki (2013 version) and by the ethical standards of the National Research Committee.

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