RESEARCH PAPER

Serum Bilirubin Level Predicts Phototherapy-Associated Gut Microbiome Disruption in Neonates with Hyperbilirubinemia

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ABSTRACT

Background: Neonatal jaundice is a frequent condition that may require phototherapy to prevent bilirubin-induced neurotoxicity.

Objective: To investigate the effects of phototherapy on intestinal probiotics in neonates with jaundice, and to identify clinical predictors of microbiota alterations following treatment

Subjects and Methods: This prospective observational research involved 33 full-term newborns who had jaundice requiring phototherapy and 35 healthy controls. Stool samples have been gathered from cases twice; prior to exposure to phototherapy and after weaning from it. Samples were withdrawn from controls on the third day of life. Gut microbiota was analyzed using metagenomic co-sequencing with quantitative PCR of Lactobacillus and Bifidobacterium.

Results: Before phototherapy, jaundiced neonates exhibited significantly lower Lactobacillus and Bifidobacterium counts compared with healthy controls ($P < 0.001$). Phototherapy has been related to a marked diminution in both probiotics (Lactobacillus: 9.00 ± 0.16 to 5.86 ± 3.15 $\log_{10}$ CFU/g; Bifidobacterium: 9.17 ± 0.14 to 6.44 ± 3.40 $\log_{10}$ CFU/g; $P < 0.001$). Higher total serum bilirubin and longer phototherapy duration were negatively correlated with probiotic counts, while gestational age and birth weight were protective. Multivariable regression identified total serum bilirubin as the strongest independent predictor of post-phototherapy microbiota reduction.

Conclusion: Phototherapy in newborns who had jaundice is related to significant disruption of beneficial gut microbiota. Elevated bilirubin levels and lower gestational age play a central role in post-treatment microbial depletion, highlighting potential implications for neonatal intestinal and metabolic health.

Keywords: Neonatal jaundice; Phototherapy; Gut microbiota; Bilirubin metabolism

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INTRODUCTION

Neonatal hyperbilirubinemia (NH) is a prevalent metabolic disorder, especially throughout the 1st week of life, affecting between eight and eleven percent of full-term infants and is even more frequently in preterm babies. In the absence of timely management, neonatal hyperbilirubinemia can advance to acute bilirubin encephalopathy and kernicterus, which may significantly impair neurological development and trigger lifelong disabilities (1).

Neonatal jaundice, marked by elevated serum bilirubin levels, leads to yellow discoloration of the skin, sclera, and mucous membranes. It is categorized into physiologic and pathologic jaundice. Pathologic neonatal jaundice is the most frequent reason for a hospital stay in the neonatal interval. The gut microbiota participates in bilirubin metabolism (2).

The practice guidelines released by the American Academy of Pediatrics (AAP) are commonly accepted for monitoring and recognizing neonates at risk for severe unconjugated hyperbilirubinemia, particularly those with preterm birth, maternofetal ABO, low birth weight, and inherited disorders (3).

Early identification and treatment of neonatal jaundice can prevent further complications. Effective treatments include blue-light phototherapy and exchange transfusion (4).

Neonatal jaundice is primarily mild to moderate; nevertheless, too elevated levels of unbound bilirubin may result in bilirubin encephalopathy by crossing the blood–brain barrier, leading to permanent damage if untreated. Currently, phototherapy is the most routine management for pathological jaundice. Phototherapy induces photoisomerization of unconjugated

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bilirubin in the skin, creating water-soluble isomers that may be excreted directly through bile and urine (5).

Phototherapy, although efficient, has been related to numerous side effects, like mother-infant separation, fever, water and electrolyte imbalances, diarrhea, skin damages, bronze baby syndrome, hematological alterations, paralytic intestinal, patent ductus arteriosus, eye damage, and disruptions in the circadian cycle (6).

Microbiota refers to the commensal, symbiotic, and pathogenic microorganisms that share the host human body. The microbiome represents all microorganisms living in the body and their genetic nature. The microbiota has begun to be recognized as a novel organ. Numerous research have examined the effects on illnesses and its application as a instrument for management (7). Gut microbiota is involved in bilirubin metabolism; nevertheless, it is uncertain whether this is affected by phototherapy (8).

Current research revealed the complex interplay among bilirubin metabolism and the gut microbiota. Bilirubin serves as a potent antioxidant and signaling molecule in humans, and its concentration-dependent effects on several microbial taxa suggest that it applies selection pressure on the gut ecosystem. The gut microbiota modulates bilirubin metabolism by changing intestinal pH, producing and activating bilirubin metabolic enzyme, and bile acids. perturbations in bilirubin handling are particularly prevalent and their possibility of neurotoxic in newborns, a concise synthesis of recent advancements is warranted (9).

This research aimed to examine the effects of phototherapy on the intestinal microbiome in neonates with hyperbilirubinemia, providing insights into potential health impacts on the neonatal environment.

MATERIALS AND METHODS

Research design and populations

This research was a prospective multi-center observational study performed on neonates with jaundice to explore the effect of phototherapy on their intestinal microbiome. Fecal samples have been gathered from 33 newborns diagnosed with neonatal jaundice (Cases) and 35 healthy age and sex matched newborns (controls). Neonates were recruited from Neonatal Intensive Care Units (NICUs) of Abu El Reish Hospital, Faculty of Medicine, Cairo University and El Demerdash Hospital , Faculty of Medicine, Ain Shams University.

Study population

Inclusion criteria: The jaundice levels met the phototherapy standards established by the American Academy of Pediatrics (3), for babies aged not more than two weeks, full-term, with gestational ages of 37–41 weeks and birth weights between 2,500 and 4,000 grams. Prior to specimen collection, neither antibiotics nor ecological preparations had been utilized, and the mother maintained a healthy weight throughout pregnancy, with no history of special medication or antibiotic use prior to, throughout, or following parturition.

Exclusion criteria: Gestational age less than 37 weeks or greater than or equal to 42 weeks, bilirubin concentration as great as the exchange standard (twenty milligrams per deciliter), pneumonia, sepsis, or additional illnesses, hereditary metabolic illnesses, severe immunodeficiency, congenital biliary malformation or other organ malformation, and drug allergies.

Methods

Detailed history was taken for neonates including gestational age, birth weight, postnatal age, feeding type, onset of jaundice. Thorough clinical examination was performed for all neonates included in the study.

Measurements included

body weight; using Seca scale for neonates approximated to the nearest 0.01 kg with a diaper only for which no correction was made, transcutaneous bilirubin using jaundicemeter and serum bilirubin assessment by colorimetric method.

Phototherapy

Newborns underwent continuous 24-hour blue light phototherapy with a wavelength of 425–475 nm, followed by a 6–8 hour rest period. Eye masks and diapers protected sensitive areas. Treatment continued based on jaundice level changes.

Specimen Collection

Two stool samples had been gathered from jaundiced newborns: the 1st sample was gathered immediately prior to phototherapy exposure, and the 2nd sample was withdrawn after weaning from phototherapy. Samples from healthy newborns had been collected on the 3rd day of life to represent normal early neonatal gut colonization. Stool samples were gathered utilizing the stool collection protocol, that involved correct hand washing prior to specimen collection, and the diaper had been opened flat. A sterile stool container with a sterile spoon was carefully opened, and approximately two millimeters of stool has been gathered. A complete information on the sample, comprising the infant's name, date, and time of sample collection, has been recorded. All samples had been promptly frozen at −80°C till further analysis

Metagenomic Sequencing:

Fecal DNA has been extracted and prepared for sequencing using various techniques, including fragmentation and PCR amplification. Sequencing has been carried out utilizing the Illumina HiSeq platform, followed by bioinformatics analysis to identify and quantify microbial species. Quantification of Lactobacillus and Bifidobacterium populations was performed via real-time quantitative PCR (qPCR) using the PerfectStart Green qPCR SuperMix (TransGen Biotech, China). The qPCR reactions were carried out on a Rotor-Gene Q thermocycler (Qiagen, Germany), while conventional PCR amplification has been performed utilzing the GeneAmp PCR System 9700 (Applied Biosystems, USA).

The primers used for Lactobacillus were:

Forward: 5′-CAGCAGTAGGGAATCTTCCAC-3′

Reverse: 5′-GGCTTTCTGGCACGTAGTTAG-3′

For Bifidobacterium, the primers were:

Forward: 5′-CGGGTGAGTAATGCGTGACC-3′

Reverse: 5′-TGATAGGACGCGACCCCA-3′

The thermal cycling conditions consisted of an initial denaturation at 95°C for 5 seconds, followed by forty cycles of denaturation at 95°C for 15 seconds and annealing/extension at 53°C for thirty seconds.

Relative bacterial counts have been determined from Ct values utilizing standard curves generated from serially diluted DNA templates and expressed as $\log_{10}$ CFU/g feces equivalents.

Ethical consideration

The research adhered strictly to the ethical standards of the 2013 Helsinki Declaration and received formal approval from the National Research Centre Ethical Committee (Approval No. 0142). Written informed consent has been gained from the parents or legal guardians of all participants prior to inclusion

Statistical Methods

All data has been examined utilizing GraphPad Prism (version 8; GraphPad Software, USA). Descriptive statistics were expressed as mean ± standard deviation (SD) or median values as appropriate. Data normality has been assessed utilizing the Shapiro–Wilk test, that revealed that several parameters were not normally distributed. Therefore, nonparametric tests were applied for subsequent analyses. Paired comparisons of bacterial counts before and after phototherapy have been carried out utilizing the Wilcoxon matched-pairs signed-rank test.

Chi-square test; used to study the correlation among 2 qualitative parameters. Fisher's Exact for chi-square when above twenty percent of the cells have expected count below five. Student T Test has been utilized to assess the statistical significance of the difference between 2 study group means. Mann Whitney test: For abnormally distributed quantitative parameters, to compare among 2 examined groups. Logistic Regression: To measures the relation among the categorical target variable and one or more independent parameters. It is beneficial for situations in which the result for a target variable may have only 2 probable types.

RESULTS

Study Population and Baseline Characteristics

This research has been performed on 33 babies diagnosed with neonatal jaundice and 35 healthy controls. Table (1) shows a statistically insignificant variances has been found among the groups in birth weight, gestational age, weight Z-score, or age in days (P>0.05), indicating group comparability. The mean postnatal age at assessment was 4.6 ± 1.2 days, and jaundice developed at a mean of 81.18 ± 30.24 hours of life. The duration of phototherapy averaged 144.73 ± 25.80 hours. Regarding bilirubin levels, the mean transcutaneous bilirubin was 18.21 ± 3.60 mg/dL, while the mean total serum bilirubin reached 20.33 ± 3.54 mg/dL

.**Table 1:** Demographic and clinical features of both jaundiced and healthy neonates.

	Jaundiced neonates (Cases) N=33	**Healthy neonates (Controls) N=35**	**Effect size**	**Test**	**P Value**	**Mean difference**	**95%CI**
Gestation age Mean ±SD	37.78±0.99	37.5±0.89	0.349	t=1.0358	0.3	0.28	-0.21-0.88
Birth weight (kg) Mean ±SD	2.96±0.27	2.93±0.39	0.103	t = 0.3308	0.7	0.034	-0.15-0.22
z score wt. Mean ±SD Median(min-mix)	-0.28±0.6 -0.28(-1.41-0.67)	-0.259±0.67 -0.435(-1.34 - 1.51)	-0.043	t = 0.0844	0.9	-0.028	-0.39-0.33
Age(days) Mean ±SD	4.6±1.2	4.85±1.53	-0.155	t = 0.6621	0.5	-0.214	-0.99-0.56
Feeding type Formula Mixed Breastfed	26(78.8%) 7(21.2%) 0(0%)	14(42.4%) 4(12.2%) 2(6.06%)	0.012	X^2=3.436	0.18	-	0.26-4.26
Onset of jaundice (hrs.) Mean ±SD	81.18±30.24	-	-	-	-	-	-
Phototherapy duration(hrs.) Mean ±SD	144.73±25.80	-	-	-	-	-	-

Transcuteous bilirubin (mg/dL) Mean ±SD	18.21±3.60	-	-	-	-	-	-
Total bilirubin (mg/dL) Mean ±SD	20.33±3.54	-	-	-	-	-	-

P value >0.05: Not significant, *P value <0.05 is statistically significant, **p<0.001 is highly significant, SD: standard deviation, X2chi square, t independent t test.

Gut Microbiota (Lactobacillus and Bifid bacteria) in jaundiced neonates before phototherapy and healthy neonates

The mean Lactobacillus count was 9.00 ± 0.16 log_{10} CFU/g in jaundiced neonates versus 10.23 ± 0.24 log_{10} CFU/g in healthy neonates, with this difference being highly statistically significant (t = 22.37, P < 0.001). Similarly, the mean Bifidobacterium count was 9.17 ± 0.14 log_{10} CFU/g among the case group compared with 10.29 ± 0.02 log_{10} CFU/g in the control group, also illustrating a highly statistically significant variance (P under 0.001).

Table 2: Gut microbiota counts (Lactobacillus and Bifidbacterium) in jaundiced neonates before phototherapy and healthy neonates.

	Jaundiced neonates before phototherapy (N=33)	**Healthy neonates (N=35)**	**Effect size**	**Test**	**P value**	**Mean difference**	**95%CI**
***Lactobacillus* counts (log_{10} CFU/g)**							
Mean ± SD	9.002± 0.16	10.23± 0.24	0.198	t = 22.37	<0.001**	1.229	1.119-1.34
***Bifidobacterium* counts (log_{10} CFU/g)**							
Mean ± SD	9.17 ± 0.14	10.29± 0.02	0.111	t = 35.43	<0.001**	1.119	1.07- 1.17

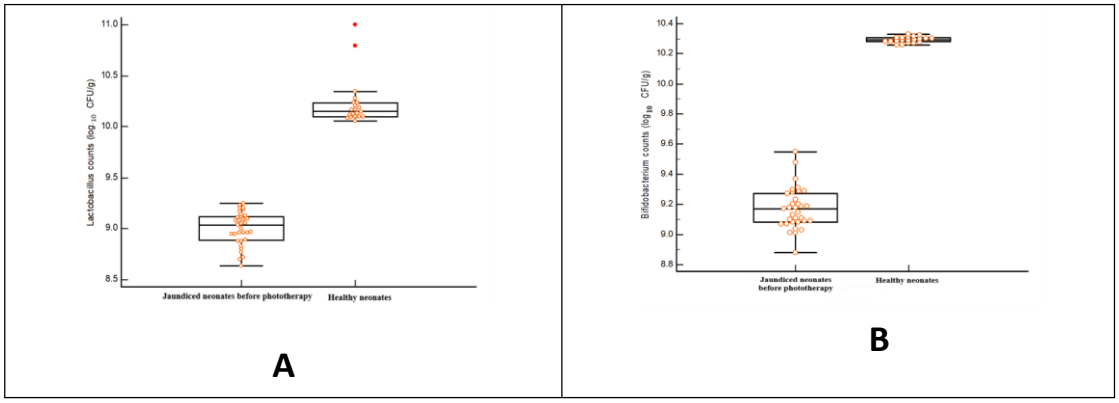

Figure 1: Box plots show the log_{10} CFU/g feces values for (A) *Lactobacillus* and (B) *Bifidobacterium* in jaundiced neonates before phototherapy and healthy neonates.

Effect of phototherapy exposure on Lactobacillus and Bifidobacterium Counts

Table (3) shows that in the jaundiced neonates' group, phototherapy has been correlated with a marked and statistically significant diminution in intestinal probiotic counts. The mean Lactobacillus count decreased from 9.00 ± 0.16 log_{10} CFU/g before phototherapy to 5.86 ± 3.15 log_{10} CFU/g after phototherapy, with this decline being highly statistically significant (P below 0.001). Similarly, the mean Bifidobacterium count showed a significant reduction from 9.17 ± 0.14 log_{10} CFU/g prior to phototherapy to 6.44 ± 3.40 log_{10} CFU/g following phototherapy (P < 0.001).

These outcomes illustrate that phototherapy exerts a significant negative impact on beneficial gut microbiota among jaundiced neonates.

Table 3: Gut microbiota counts (Lactobacillus and Bifid bacteria) in jaundiced neonates before and after phototherapy.

	Jaundiced neonates (N=33)	**Effect**	**Test**	**P value**	**Mean**	**95%CI**

	Before phototherapy	After phototherapy	size			difference	
***Lactobacillus* counts (log_{10} CFU/g)**							
Mean ± SD	9.002± 0.16	5.86±3.15	1.02	Paired t = 5.95	<0.001**	3.14	2.07-4.22
Bifidobacterium counts (log_{10} CFU/g)							
Mean ± SD	9.17 ± 0.14	6.44± 3.4	0.81	Paired t = 4.68	<0.001**	2.726	1.54-3.91

The heatmaps in figure (2) illustrate the distribution and density of paired before–after measurements of intestinal microbiota in the cases group. For both Lactobacillus and Bifidobacteria, higher color intensity (green) indicates a greater concentration of subjects within specific before–after value ranges, whereas red denotes sparse or absent observations. The plots demonstrate clustering toward lower post-phototherapy counts compared with baseline, indicating an overall reduction in bacterial load following phototherapy, with interindividual variability evident from the spread of data across bins.

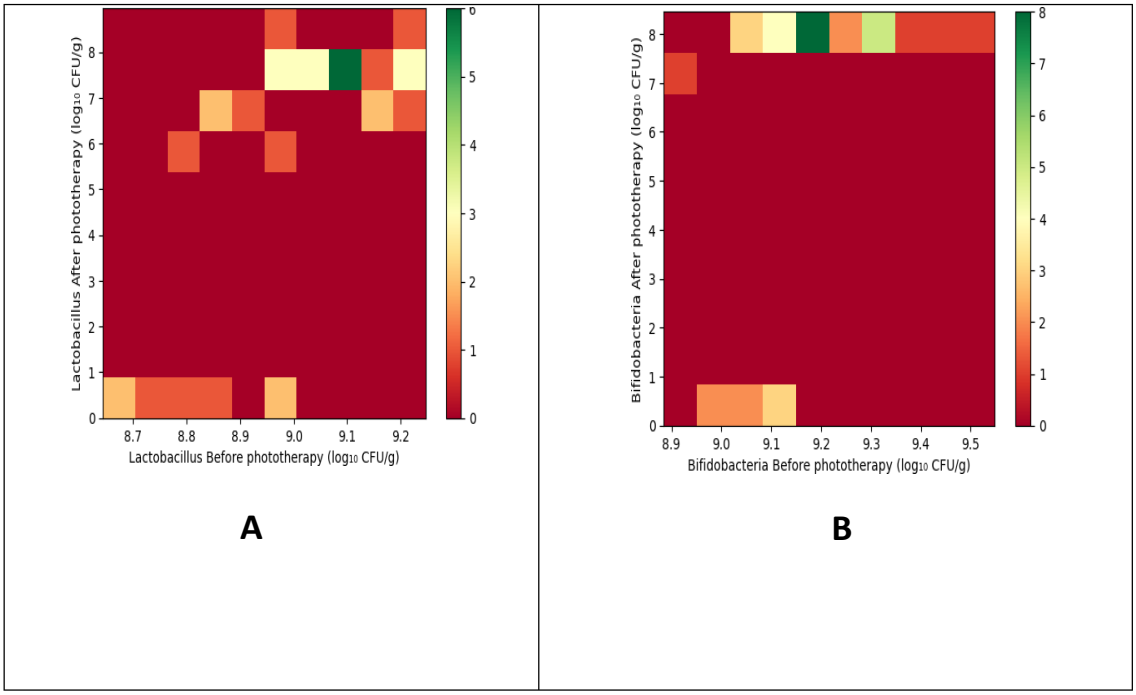

Figure 2: Heatmap Showing A) Lactobacillus counts before and after phototherapy in neonates with Jaundice, B) Bifidobacterium Counts before and after phototherapy in neonates with Jaundice

Correlation analysis & regression

Correlation analysis demonstrated that gestational age was positively related to both Lactobacillus (r equal 0.374, P equal 0.032) and Bifidobacterium counts before phototherapy (r equal 0.617, P below 0.001). Infant weight illustrated a strong positive relation to both bacterial populations (r equal 0.617, P < 0.001 for each). In contrast, total serum bilirubin was inversely correlated with Lactobacillus count (r equal −0.682, P under 0.001), whereas non-significant correlation has been found with Bifidobacterium count (r equal −0.154, P equal 0.393). Regarding the onset of jaundice, no significant association has been detected with Lactobacillus count (r equal −0.270, P equal 0.128); however, a significant positive association has been observed with Bifidobacterium count (r equal 0.617, P below 0.001).

Table 4: Correlations between microbiota counts before phototherapy & gestational age, weight, total serum bilirubin, onset of jaundice

Variables	Lactobacillus count before phototherapy		Bifidobacterium count before phototherapy	
	r	P-value	r	P-value
Gestational age (weeks)	0.374	0.032	0.617	<0.001**
Infant weight (Kg)	0.617	<0.001**	0.617	<0.001**

Total serum bilirubin (mg/dL)	-0.682	<0.001**	-0.154	0.393
Onset of jaundice	-0.270	0.128	0.617	<0.001**

R: Correlation Coefficient

Correlation analysis after phototherapy revealed that phototherapy duration was significantly and inversely related to both Lactobacillus (r = −0.385, P = 0.027) and Bifidobacterium counts (r = −0.397, P = 0.022). Total serum bilirubin showed a strong negative association with Lactobacillus count (r = −0.593, P = 0.0003) and a moderate negative association with Bifidobacterium count (r = −0.424, P = 0.014).

In contrast, birth weight was positively correlated with both bacterial populations, with significant associations observed for Lactobacillus (r = 0.364, P = 0.037) and Bifidobacterium (r = 0.469, P = 0.006). Infant age illustrated a significant inverse relation to both Lactobacillus (r = −0.375, P = 0.0315) and Bifidobacterium counts (r = −0.404, P = 0.0197). No significant associations have been found among feeding type and either Lactobacillus (r = 0.275, P = 0.121) or Bifidobacterium counts (r = 0.288, P = 0.104) after phototherapy.

Table 5: Correlations between microbiota counts after phototherapy and duration of photo, total serum bilirubin, feeding type, and infant age.

Variables	**Lactobacillus count after phototherapy**		**Bifidobacterium count after phototherapy**	
	r	**P-value**	**r**	**P-value**
Phototherapy duration	-0.385	0.027*	-0.397	0.022*
Total serum bilirubin	-0.593	0.0003**	-0.424	0.0140*
Birth weight (kg)	0.364	0.037*	0.469	0.006*
Feeding type	0.275	0.121	0.288	0.104
Infant age (days)	-0.375	0.0315*	-0.404	0.0197*

Univariate linear regression analysis has been performed to identify predictors of decreased gut microbiota counts after phototherapy. For Lactobacillus counts, significant positive associations were observed with gestational age (β = 0.369, P = 0.035) and birth weight (β = 0.393, P = 0.024), whereas significant negative associations were found with phototherapy duration (β = −0.385, P = 0.027), transcutaneous bilirubin (β equal −0.715, P under 0.001), and total serum bilirubin (β equal −0.670, P below 0.001). Other variables, involving infant age, onset of jaundice, and feeding type, weren't significantly related.

Similarly, for Bifidobacterium counts, significant positive associations were observed with gestational age (β = 0.439, P = 0.011) and birth weight (β = 0.386, P = 0.026), while significant negative associations were identified with phototherapy duration (β = −0.397, P = 0.022), transcutaneous bilirubin (β = −0.673, P < 0.001), and total serum bilirubin (β = −0.625, P < 0.001). Other predictors, including infant age, onset of jaundice, and feeding type, were not statistically significant.

Table 6: Univariate linear regression analysis for predictors of decreased gut microbiota counts after phototherapy.

Predictor	**Lactobacillus count after phototherapy**		**Bifidobacterium count after phototherapy**	
	β (Standardized)	**P-value**	**β (Standardized)**	**P-value**
Gestation age (weeks)	0.369	0.035*	0.439	0.011*
Age (days)	-0.320	0.069	-0.278	0.117
Birth weight (kg)	0.393	0.024*	0.386	0.026*
Onset of jaundice (hrs)	-0.303	0.086	-0.260	0.145
Phototherapy duration (hrs)	-0.385	0.027*	-0.397	0.022*
Transcutaneous bilirubin (mg/dL)	-0.715	<0.001**	-0.673	<0.001**
Total bilirubin (mg/dL)	-0.670	<0.001**	-0.625	<0.001**
Feeding type	0.275	0.121	0.288	0.104

B (Standardized): standardized regression coefficient

Multivariable linear regression analysis has been performed to recognize independent predictors of gut microbiota counts after phototherapy. For Lactobacillus counts, the model explained 45.4 percent of the variance (Adjusted R^2 = 0.454, F (4,41) = 7.66, P < 0.001). Among the predictors, total serum bilirubin was the strongest independent factor associated with decreased Lactobacillus counts (β equal −0.685, P below 0.001). Gestational age was positively related to Lactobacillus counts (β equal 0.294, P equal 0.047), while phototherapy duration and birth weight were not statistically significant.

Similarly, for Bifidobacterium counts, the model clarified 44.7% of the variance (Adjusted R^2 = 0.447, F(4,48) = 7.47, P < 0.001). Total serum bilirubin again emerged as the strongest predictor of decreased counts (β = −0.611, P = 0.001), and gestational age was positively associated (β

= 0.380, P = 0.013). Phototherapy duration and birth weight were not independently significant. Collinearity diagnostics indicated no problematic multicollinearity (VIF < 5). These findings suggest that higher bilirubin levels and lower gestational age are the most important factors contributing to reduced gut microbiota counts after phototherapy

Table 7: Multivariable linear regression analysis for predictors of decreased gut microbiota counts after phototherapy.

	B (Unstandardized)	SE	β (Standardized)	t	P-value	95% CI for B	VIF
Dependent variable: Lactobacillus count after phototherapy							
Phototherapy duration	−0.006	0.022	−0.046	−0.260	0.797	−0.050 to 0.039	1.85
Total serum bilirubin	−0.608	0.152	−0.685	−4.005	<0.001	−0.919 to −0.297	1.71
Birth weight (kg)	−1.833	2.239	−0.159	−0.819	0.42	−6.420 to 2.754	2.21
Gestation age (weeks)	0.933	0.449	0.294	2.079	0.047	0.014 to 1.853	1.18
R^2 / Adjusted R^2	0.522/ 0.454						
F (df)	F(4,41)=7.66						
Dependent variable: Bifidobacterium count after phototherapy							
Phototherapy duration	−0.014	0.024	−0.103	−0.579	0.568	−0.062 to 0.035	1.85
Total serum bilirubin	−0.587	0.165	−0.611	−3.549	0.001	−0.926 to −0.248	1.71
Birth weight (kg)	−2.369	2.438	−0.190	−0.971	0.34	−7.363 to 2.626	2.21
Gestation age (weeks)	1.302	0.489	0.38	2.662	0.013	0.300 to 2.303	1.18
R^2 / Adjusted R^2	0.516/ 0.447						
F (df)	F(4,48)=7.47						

R^2: Coefficient of Determination
Df: degrees of freedom, F: F statistic for overall model significance

DISCUSSION

Neonatal hyperbilirubinemia is a clinical disorder characterized by multiple factors that inhibit the liver's capacity to produce, combine, absorb, and excrete bilirubin, resulting in neonatal jaundice. Jaundice appears in the early neonatal duration, based on its etiology. Jaundice results from the deposition of indirect bilirubin in the skin, which turns the skin yellow or light orange. In obstructive jaundice (direct bilirubin), the skin becomes a greenish or muddy yellow (10).

Around sixty percent of term newborns and eighty percent of preterm babies had neonatal jaundice during their 1st week of life. The most prevalent etiology of hyperbilirubinemia is physiologic jaundice, identified by rejecting other etiologies such as hemolysis, infection, and metabolic disorders (1-3). In two percent of term newborns, the concentration of bilirubin can reach to twenty milligrams per deciliter, needing therapeutic intervention; if left untreated, it may result in bilirubin-induced neurologic dysfunction and chronic neurological damage (11).

Regarding demographic and clinical features of healthy and jaundiced neonates, a statistically insignificant variances has been found among the 2 groups in birth weight, gestational age, weight Z-score, or age in days (P>0.05), indicating group comparability. The mean postnatal age at assessment was 4.6 ± 1.2 days, and jaundice developed at a mean of 81.18 ± 30.24 hours of life. The duration of phototherapy averaged 144.73 ± 25.80 hours. Regarding bilirubin levels, the mean transcutaneous bilirubin was 18.21 ± 3.60 mg/dL, while the mean total serum bilirubin reached 20.33 ± 3.54 mg/dL.

Regarding comparison between gut microbiota (Lactobacillus and Bifid bacteria) in jaundiced neonates before phototherapy and healthy neonates, findings showed that the mean Lactobacillus count was 9.00 ± 0.16 $\log_{10}$ CFU/g in cases versus 10.23 ± 0.24 $\log_{10}$ CFU/g in controls, with this difference being highly statistically significant (t = 22.37, P < 0.001). Similarly, the mean Bifidobacterium count was 9.17 ± 0.14 $\log_{10}$ CFU/g among cases compared with 10.29 ± 0.02 $\log_{10}$ CFU/g in the control group, also illustrating a highly statistically significant variance (P below 0.001).

When considering increased bilirubin level impacts on neonatal gut microbiota counts, our outcomes are consistent with the study of Zhang et al., who examined 69 neonates to recognize whether gut microbiota composition may distinguish newborns with hyperbilirubinemia (12). Their study reported that neonates with hyperbilirubinemia had a significantly lower relative abundance of Lactobacillus compared with healthy controls in 16S rRNA analyses of meconium samples, with decreased diversity metrics also observed in case groups. As well,

our study agreed with Akagawa et al., who revealed the gut microbiota features in 26 newborns with jaundice. Their study found that Bifidobacterium-related taxa were depleted in neonates with jaundice, and that the reduction of Bifidobacteria was one of the most consistent signatures of gut dysbiosis associated with hyperbilirubinemia (13), supporting the lower Bifidobacteria counts seen in our cohort.

Our results are on the contrary to Chen et al., who described the critical microbiota signatures for neonatal hyperbilirubinemia and focused on the underlying pathogenic mechanism. They demonstrated that an insignificant variance has been found in overall alpha-diversity or Bifidobacterium abundance between jaundiced and non-jaundiced infants, indicating that microbial dysbiosis is not universal across all populations or sampling time points check this comment on original paper (14). In agreement with Wu et al., who evaluated the useful alters in gut microbiota following phototherapy for neonatal hyperbilirubinemia. Their study reported that neonates with jaundice exhibited relatively high baseline levels of beneficial bacteria before phototherapy, suggesting that hyperbilirubinemia alone does not necessarily disrupt early gut microbial colonization. Their study also demonstrated that Lactobacillus and Bifidobacterium constituted more than 60% of total bacterial abundance prior to treatment (6).

Regarding bacterial count in jaundiced neonates (Lactobacillus and Bifid bacteria) before and after exposure to phototherapy, we documented that phototherapy was associated with a marked and statistically significant reduction in intestinal probiotic counts. The mean Lactobacillus count decreased from 9.00 ± 0.16 $\log_{10}$ CFU/g before phototherapy to 5.86 ± 3.15 $\log_{10}$ CFU/g after phototherapy, with this decline being highly statistically significant ($P < 0.001$). Similarly, the mean Bifidobacterium count showed a significant reduction from 9.17 ± 0.14 $\log_{10}$ CFU/g prior to phototherapy to 6.44 ± 3.40 $\log_{10}$ CFU/g following phototherapy ($P < 0.001$). These outcomes illustrate that phototherapy exerts a significant negative impact on beneficial gut microbiota among jaundiced neonates.

Supporting our findings, Fan et al., reported that both Bifidobacterum and Lactobacillus decreased significantly after 24 and 48 hours of phototherapy in term neonates ($P < 0.05$ for both taxa). This study also linked changes in these probiotics to shifts in metabolism, involving short-chain fatty acids and bile acids, suggesting a broader impact of phototherapy on microbial function and metabolites (1). Similarly, Yan et al., observed a significant alteration in microbial diversity indices, with a reduction in Shannon diversity scores after phototherapy compared to baseline values ($P < 0.001$). These findings suggest that phototherapy itself, rather than jaundice alone, may be responsible for microbial shifts (15).

On the other hand, Zhang et al., who assessed the benefit of treatment for hyperbilirubinemia with phototherapy, found that microbial changes after phototherapy may depend on baseline microbiome composition, antibiotic exposure, feeding practice, and timing of sample collection, meaning that simple measures of total Lactobacillus or Bifidobacterium may not capture the nuanced restructuring that occurs (16).

Concerning correlations between bacterial count before phototherapy & gestational age, weight, total serum bilirubin, onset of jaundice findings showed that gestational age was positively related to both Lactobacillus (r equal 0.374, P equal 0.032) and Bifidobacterium counts before phototherapy (r equal 0.617, P under 0.001). Infant weight illustrates a strong positive association with both bacterial populations (r equal 0.617, P below 0.001 for each). In contrast, total serum bilirubin was inversely correlated with Lactobacillus count (r equal −0.682, $P < 0.001$), while no significant association was observed with Bifidobacterium count (r equal −0.154, P equal 0.393). Regarding the onset of jaundice, non-significant association was detected with Lactobacillus count (r equal −0.270, P equal 0.128); however, a significant positive association was observed with Bifidobacterium count (r equal 0.617, P below 0.001).

Our outcomes are in concurrent with Li et al., who evaluated progression of gut microbiome in 69 preterm neonates throughout the 1st month. Their study reported that gestational age and infant growth influence early gut colonization patterns. For example, longitudinal microbiome research in neonates has shown that gestational age correlates with increasing microbial diversity and colonization of useful taxa like Bifidobacterium and Lactobacillus over time, particularly in term infants compared with preterm counterparts (17). AS well, our study agreed with Rahimi et al., their study found that the positive association between birth weight and probiotic counts is linked to greater microbial stability and faster restoration of beneficial taxa following clinical interventions (18).

However, Zhang et al., reported that correlations between Lactobacillus or Bifidobacterium levels and clinical variables such as serum bilirubin or growth parameters were not statistically robust, highlighting variability based on study design and cohort characteristics (16).

Correlations between bacterial count after phototherapy and duration of photo, total serum bilirubin, feeding type, and infant age findings showed that phototherapy duration was significantly and inversely related to both Lactobacillus ($r = −0.385$, $P = 0.027$) and Bifidobacterium counts ($r = −0.397$, $P = 0.022$). Total serum bilirubin showed a strong negative association with Lactobacillus count ($r = −0.593$, $P = 0.0003$) and a moderate negative association with Bifidobacterium count ($r = −0.424$, $P = 0.014$). In contrast, birth weight was positively correlated with both bacterial populations, with significant associations observed for Lactobacillus (r equal 0.364, P equal 0.037) and Bifidobacterium (r equal 0.469, P equal 0.006). Infant age illustrated a significant inverse

correlation with both Lactobacillus (r = −0.375, P = 0.0315) and Bifidobacterium counts (r equal −0.404, P = 0.0197). Non-significant associations have been found among feeding type and either Lactobacillus (r = 0.275, P = 0.121) or Bifidobacterium counts (r = 0.288, P = 0.104) after phototherapy.

Our results reinforce those of Fan et al., who stated that longer phototherapy duration was significantly associated with reductions in Bifidobacterium and Lactobacillus abundance, alongside alterations in bile acid metabolism. The progressive decline in probiotic taxa was proportional to phototherapy exposure time (1), supporting the inverse correlations observed in our results.

In contrast with You et al., who demonstrated the relation between gut microbiota and neonatal pathologic jaundice. They documented feeding type as a major determinant of post-phototherapy microbiota composition, particularly highlighting higher Bifidobacterium abundance in exclusively breastfed neonates (19). However, these studies often evaluated microbiota at later time points or excluded phototherapy duration as a confounding variable, which may explain the discrepancy with our findings.

Regarding univariate linear regression analysis for predictors of decreased gut microbiota counts after phototherapy findings showed that for Lactobacillus counts, significant positive associations were observed with gestational age (β = 0.369, P = 0.035) and birth weight (β = 0.393, P = 0.024), whereas significant negative associations were found with phototherapy duration (β = −0.385, P = 0.027), transcutaneous bilirubin (β equal −0.715, P below 0.001), and total serum bilirubin (β equal −0.670, P below 0.001). Other variables, including infant age, onset of jaundice, and feeding type, were not significantly associated. Similarly, for Bifidobacterium counts, significant positive associations were observed with gestational age (β equal 0.439, P equal 0.011) and birth weight (β = 0.386, P = 0.026), while significant negative associations were identified with phototherapy duration (β = −0.397, P = 0.022), transcutaneous bilirubin (β = −0.673, P < 0.001), and total serum bilirubin (β = −0.625, P < 0.001). Other predictors, including infant age, onset of jaundice and feeding type, were not statistically significant.

Similarly, Fan et al., reported that phototherapy duration and bilirubin levels were independently associated with reductions in Bifidobacterium and Lactobacillus abundance in neonates with hyperbilirubinemia. This study concluded a significant negative relationship between phototherapy exposure time and probiotic counts (P < 0.05) (1), supporting the negative β-coefficients observed in the present regression model.

In contrast with You et al., study which reported feeding type as a significant predictor of post-phototherapy microbiota composition, particularly noting higher Bifidobacterium abundance in exclusively breastfed infants (19). However, these studies often used multivariate sequencing-based approaches and evaluated later post-treatment time points, which may explain why feeding type was not a significant predictor in our univariate regression model.

In the current work, multivariable linear regression analysis for predictors of decreased gut microbiota counts after phototherapy findings demonstrated that For Lactobacillus counts, the model explained 45.4 percent of the difference (Adjusted R^2 = 0.454, F (4,41) = 7.66, P < 0.001). Among the predictors, total serum bilirubin was the strongest independent factor associated with decreased Lactobacillus counts (β = −0.685, P < 0.001). Gestational age was positively associated with Lactobacillus counts (β = 0.294, P = 0.047), while phototherapy duration and birth weight were not statistically significant. Similarly, for Bifidobacterium counts, the model explained 44.7% of the variance (Adjusted R^2 = 0.447, F (4,48) = 7.47, P < 0.001). Total serum bilirubin again emerged as the strongest predictor of decreased counts (β = −0.611, P = 0.001), and gestational age was positively associated (β = 0.380, P = 0.013). Phototherapy duration and birth weight were not independently significant. Collinearity diagnostics indicated no problematic multicollinearity (VIF < 5). These findings suggest that higher bilirubin levels and lower gestational age are the most important factors contributing to reduced gut microbiota counts after phototherapy.

Zhou et al., studied the composition of gut microbiota in 40 cases with neonatal cholestasis (NC) and breast milk jaundice (BMJ). They reported a significant decrease in Bifidobacterium abundance, that was negatively correlated with bilirubin levels (20), supporting the finding that higher bilirubin predicts lower probiotic counts after treatment. As well, our study agreed with Lin et al., who investigated the correlation among the gut microbiota and neonatal hyperbilirubinemia, assessing the potential and underlying mechanisms of Bifidobacterium in managing the condition. They found that more mature infants tend to develop a more robust and stable gut microbiome. Neonates with higher gestational age typically show increased abundance of useful bacteria like Bifidobacterium and Lactobacillus during the early postnatal period (21).

Also, our study agreed with Kamel et al., who revealed that although breast milk is an important source of beneficial bacteria, its influence on neonatal gut microbiota is modulated by multiple perinatal factors, including delivery mode, environmental exposure and early clinical interventions. Their study demonstrated that the transmission of Lactobacillus and Bifidobacterium from breast milk to the neonatal gut was not uniform across all infants and was less pronounced in neonates exposed to medical interventions, suggesting that external factors may attenuate the effect of feeding type alone (22).

Finally, contrasting with our results, Su et al., evaluated the importance of the gut microbiota in the mechanism of jaundice. Their study reported that the direct role of certain probiotic genera (like Lactobacillus) in bilirubin

metabolism, instead characterizing their roles as secondary or context-dependent compared to taxa such as Escherichia or Clostridium (23).

CONCLUSION AND RECOMMENDATIONS

This current study shows that phototherapy in neonates with jaundice is related to a significant decrease in beneficial intestinal probiotics, particularly Lactobacillus and Bifidobacterium. Although baseline probiotic levels were relatively preserved before treatment, jaundiced neonates had lower counts than healthy controls, and phototherapy led to a marked further decline. Higher bilirubin levels and longer phototherapy duration were strongly associated with decreased probiotic counts, while greater gestational age and birth weight were protective factors. Multivariable analysis identified total serum bilirubin as the strongest independent predictor of post-phototherapy probiotic reduction. Feeding type did not significantly influence gut microbiota in the early post-treatment period.

Oral probiotics are often administered to reduce jaundice, but many lack scientific evidence. To address this, metagenomic sequencing and metabonomics are being used to investigate changes in probiotic bacteria in the neonatal gut following phototherapy and the relationship between probiotic alterations and metabolic processes.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the research, encompassing study conception and design, information collection, analysis and interpretation of outcomes, draft manuscript, reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest was declared

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