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Original Article

Effectiveness of Probiotics in Treating Jaundice in Preterm Low Birth Weight Infants

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ABSTRACT

Background: Neonatal jaundice is a common condition in low birth weight (LBW) infants (birth weight <2500 g) that, if left untreated, may lead to severe complications such as kernicterus. This study aimed to evaluate the efficacy of the probiotic Pedilact as an adjunctive therapy to phototherapy in reducing serum bilirubin levels and the duration of phototherapy in preterm LBW infants with jaundice.

Methods: In this double-blind randomized clinical trial, 72 preterm LBW infants admitted to the Neonatal Intensive Care Unit (NICU) of Imam Reza Hospital, Mashhad, with clinical jaundice were randomly assigned into two equal groups. The intervention group (n=36) received 5 drops (approximately 0.25 mL) of Pedilact daily for three consecutive days alongside standard phototherapy, while the control group (n=36) received 5 drops of distilled water as placebo. Serum bilirubin levels were measured at 24, 48, and 72 hours post-treatment initiation.

Results: The percentage of infants with bilirubin <10 mg/dL in the probiotic group at 24, 48, and 72 hours was 44.4%, 69.4%, and 75%, respectively, compared to 25%, 22.2%, and 16.7% in the control group. Mean bilirubin levels were also significantly lower in the probiotic group at all time points (P<0.001 for all comparisons). The duration of phototherapy was significantly shorter in the probiotic group (3.87 ± 0.50 days) compared to the control group (4.32 ± 1.10 days; P=0.049).

Conclusion: Pedilact probiotic supplementation as an adjunct to phototherapy significantly accelerates bilirubin reduction in preterm LBW infants with neonatal jaundice, potentially reducing phototherapy duration and hospital stay. These findings support the use of probiotics as a safe and effective complementary treatment in managing neonatal hyperbilirubinemia.

Keywords: Low birth weight, Neonatal jaundice, Phototherapy, Preterm, Probiotics

Introduction

Hyperbilirubinemia in newborns, marked by increased serum bilirubin, manifests in roughly 60% of term and 80% of preterm neonates during the initial postnatal week (1). This disorder exhibits heightened incidence and intensity among infants with birth weights below 2500 g, attributable to immature liver function and intestinal maturation, thereby elevating susceptibility to grave sequelae such as bilirubin encephalopathy and enduring neurodevelopmental deficits (2). Conventional management relies on phototherapy, which facilitates bilirubin reduction via photochemical conversion to polar, renally excretable isomers (3). Nevertheless, phototherapy is associated with

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adverse effects, including thermoregulatory instability, fluid loss, and compromised parental–neonatal attachment, often hindering lactation establishment (4).

Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, are under investigation as supplementary interventions; they enhance intestinal motility and reduce bilirubin enterohepatic circulation, thereby promoting fecal bilirubin excretion (5,6). Prior clinical trials have reported variable outcomes concerning their therapeutic value (7,8). Accordingly, this investigation assesses the impact of Pedilact—a probiotic formulation comprising *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, and *Bifidobacterium infantis*—when administered alongside phototherapy, on serum bilirubin decline and treatment duration in preterm low-birth-weight neonates presenting with jaundice at Imam Reza Hospital, Mashhad.

The primary objective of this study was to determine whether Pedilact probiotic supplementation, when administered alongside standard phototherapy, produces a significant reduction in serum bilirubin levels at 24, 48, and 72 hours and decreases the duration of phototherapy in preterm low-birth-weight neonates with hyperbilirubinemia.

Methods

This randomized controlled, double-blinded clinical trial was conducted between 2023 and 2024 on preterm neonates admitted to the Neonatal Intensive Care Unit (NICU) of Imam Reza Hospital, affiliated with Mashhad University of Medical Sciences.

Inclusion Criteria

Preterm neonates (gestational age <37 weeks) weighing 1000–2500 g at birth, aged less than 7 days, hospitalized in the NICU for hyperbilirubinemia, exhibiting no evidence of infection, cardiac, gastrointestinal, renal, or respiratory pathology requiring ventilatory support, and lacking congenital malformations or hemolytic etiologies of jaundice; parental informed consent was mandatory.

Exclusion Criteria

Emergence of sepsis, revocation of parental consent, or designation as term neonates (gestational age ≥37 weeks).

Intervention group (n=36): Received 5 drops (approximately 0.25 mL) of Pedilact (manufactured by Zist Takhmir Pharmaceutical Company, Iran) daily for 3 days alongside standard phototherapy.

Control group (n=36): Received 5 drops of distilled water daily for 3 days alongside standard phototherapy.

Serum bilirubin concentrations were assessed prior to intervention initiation and subsequently at 24, 48, and 72 hours thereafter using the photometric technique on the Prestige 24i autoanalyzer (Japan). Venous blood specimens were centrifuged to isolate serum. Assays employed commercial reagent kits from Pars Azmun Company (Iran), relying on targeted biochemical reactions and spectrophotometric absorbance. All procedures adhered to manufacturer protocols and incorporated internal quality control measures (9).

A standardized phototherapy protocol was applied uniformly to both groups. All infants received phototherapy using the Tepko SMG LED phototherapy unit, which delivers blue light within the 430–490 nm wavelength range. The irradiance level (30–35 $\mu W/cm^2/nm$) was measured prior to treatment and every 12 hours using a radiometer. The distance between the device and the infant was maintained at 35–40 cm, with full-body exposure except for protective eye covering. Phototherapy was initiated based on AAP guidelines for preterm infants weighing <2500 g and discontinued once bilirubin levels fell below 10 mg/dL with a stable downward trend. This standardized protocol ensured consistent treatment conditions across both groups.

The study was double-blinded. The probiotic and placebo preparations were packaged in identical containers with identical appearance, color, and smell by a pharmacist not involved in patient care. Parents, attending physicians, nurses, and laboratory personnel were all blinded to group allocation. The randomization code was kept sealed and only broken after data analysis was completed.

The required sample size was calculated based on a previous study by Eghbalian et al. (10), assuming 80% power, an alpha level of 0.05, and an expected effect size of 1.5 mg/dL difference in bilirubin reduction between groups. The calculated sample size was 33 neonates per group. To account for an anticipated dropout rate of approximately 10%, 36 neonates were enrolled in each group.

Data were analyzed using SPSS version 27 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was employed to assess the normality of data

distribution. Normally distributed continuous variables were compared using independent t-tests, while categorical variables were evaluated with Chi-square or Fisher exact tests. Changes over time and differences between groups were analyzed using mixed-effects models with time as a within-subjects factor and group as a between-subjects factor. A P-value less than 0.05 was considered statistically significant (11).

Ethical approval

The study protocol was reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences (Ethics ID: IR.MUMS.MEDICAL.REC.1403.342). Written informed consent was obtained from the parents or legal guardians of all participants prior to enrollment. The trial was registered retrospectively with the Iranian Registry of Clinical Trials (registration code: IRCT20250103064264N1).

Results

A total of 72 preterm LBW infants were enrolled and randomized (36 per group). During the study, 8 infants were lost to follow-up (3 in the probiotic group and 5 in the control group), leaving 64 infants for the final analysis: 33 in the probiotic group and 31 in the control group. The reasons for dropout included transfer to another hospital (n=4), parental withdrawal of consent (n=2), and development of sepsis (n=2).

Demographic and baseline characteristics of the infants and their mothers are summarized in Tables 1 and 2. There were no significant differences between the groups regarding infant age (P=0.48), sex (P>0.99), birth weight (1919.39 ± 445.33 g in the probiotic group vs. 1913.06 ± 412.10 g in the control group; P=0.953), or infant blood group (P=0.489). In the control group, B+ was the most common blood type (16 infants, 51.6%), while A-, B-, and AB- were the least common (1 infant each, 3.2%). In the probiotic group, B+ was also predominant (24 infants, 72.7%), with no infants in B- or O- groups (0%).

There were no significant differences between the groups in the use of antibiotics or corticosteroids during hospitalization (P>0.99) or delivery type (P=0.489), with cesarean being the most frequent in both groups (87.1% in control vs. 90.9% in probiotic). All infants were born before 37 weeks of gestation, and the mean gestational age did not differ significantly between groups (P=0.31). Regarding maternal blood groups, B+ was the most common in both groups (48.4% in control and 36.4% in probiotic), followed by AB+ (38.7% in control). Statistical analysis indicated no significant difference in maternal blood group distribution between the groups (P=0.40).

Overall, these baseline similarities indicate

Table 1. newborn outcomes in the control group and the probiotic intervention group

Variable	Control (n=36)	Intervention (n=36)	P value
Birth Weight (g), mean ± SD	1913.06 ± 412.10	1919.39 ± 445.33	0.953
Hemoglobin (g/dl), mean ± SD	18.10 ± 2.11	17.14 ± 1.95	
Hemoglobin <14	1 (2.8%)	2 (5.6%)	0.063
Hemoglobin 14–21	33 (91.7%)	34 (94.4%)	
Hemoglobin >21	2 (5.6%)	0 (0%)	
Hematocrit (%), mean ± SD	49.24 ± 5.45	49.29 ± 11.04	
Hematocrit <40	2 (5.6%)	2 (6.3%)	
Hematocrit 40–60	30 (83.3%)	27 (84.4%)	
Hematocrit >60	4 (11.1%)	3 (9.4%)	
Bilirubin Baseline	11.57 ± 3.36	11.02 ± 3.64	0.542
Bilirubin 24 hr	11.81 ± 3.01	9.13 ± 3.44	<0.001
Bilirubin 48 hr	12.09 ± 2.53	7.95 ± 2.68	<0.0001
Bilirubin 72 hr	11.70 ± 2.58	6.79 ± 2.01	<0.0001
Bilirubin Categories 24 hr <10	9 (25%)	16 (44.4%)	
Bilirubin Categories 24 hr 10–15	20 (55.6%)	14 (38.9%)	0.000
Bilirubin Categories 24 hr ≥15	7 (19.4%)	6 (16.7%)	
Bilirubin Categories 48 hr <10	8 (22.2%)	25 (69.4%)	
Bilirubin Categories 48 hr 10–15	21 (58.3%)	8 (22.2%)	<0.0001
Bilirubin Categories 48 hr ≥15	7 (19.4%)	3 (8.3%)	
Bilirubin Categories 72 hr <10	6 (16.7%)	27 (75%)	
Bilirubin Categories 72 hr 10–15	23 (63.9%)	9 (25%)	<0.0001
Bilirubin Categories 72 hr ≥15	7 (19.4%)	0 (0%)	

that randomization was successful and that any observed differences in outcomes are likely attributable to the intervention rather than pre-existing differences between groups.

Hemoglobin levels in the enrolled infants were generally within the range of 14–21 g/dL. The mean hemoglobin was 18.10 ± 2.11 g/dL in the control group and 17.14 ± 1.95 g/dL in the probiotic group, with no statistically significant difference between groups (P=0.063).

Baseline serum bilirubin levels prior to the intervention were comparable between groups (P=0.542). However, at 24 hours post-intervention, infants in the probiotic group had significantly lower mean bilirubin levels compared to the control group (P<0.001). At 48 hours, 25 infants (69.4%) in the probiotic group had bilirubin levels <10 mg/dL, whereas only 8 infants (22.2%) in the control group achieved this level. Conversely, bilirubin levels between 10–15 mg/dL were observed in 21 infants (58.3%) in the control group compared to 8 infants (22.2%) in the probiotic group (P<0.001).

At 72 hours, 27 infants (75%) in the probiotic group had bilirubin <10 mg/dL, compared to only 6 infants (16.7%) in the control group. The most frequent bilirubin range in the control group was 10–15 mg/dL (23 infants, 63.9%) versus 9 infants (25%) in the probiotic group (P<0.001). Mean serum bilirubin at 48 hours was 7.95 ± 2.68 mg/dL in the probiotic group and 12.09 ± 2.53 mg/dL in the control group, and at 72 hours, 6.79 ± 2.01 mg/dL versus 11.70 ± 2.58 mg/dL, respectively. These differences were statistically significant (P<0.001), indicating a faster and greater reduction in bilirubin levels in the probiotic group.

The duration of phototherapy was also significantly shorter in the probiotic group (3.87 ± 0.50 days) compared to the control group (4.32 ± 1.10 days; P=0.049).

Analysis of serum bilirubin, while adjusting for baseline levels, showed a significant main effect of time on bilirubin changes (P<0.001) and a significant effect of group (P<0.001). A significant interaction between time and group was observed (P<0.001), indicating that differences between the probiotic and control groups were statistically significant at all measured time points (24 hours, 48 hours, and 72 hours; P<0.001 for all comparisons).

Adverse Events

No serious adverse events were reported in either group. Mild gastrointestinal symptoms (transient loose stools) were observed in 2 infants (6.1%) in the probiotic group and 1 infant (3.2%) in the control group, with no significant difference between groups (P=0.58). No cases of probiotic-related sepsis or feeding intolerance requiring intervention were observed.

Continuous variables, including birth weight, hemoglobin, hematocrit, and bilirubin at different time points, are presented as mean ± SD, while categorical variables are presented as counts and percentages. There were no significant differences in birth weight, hemoglobin, or hematocrit between the groups. Bilirubin levels at 24, 48, and 72 hours were significantly lower in the probiotic group compared with controls (P < 0.001). Categorical analysis of bilirubin also shows a higher proportion of newborns with lower bilirubin in the probiotic group over time. Overall, the table suggests that probiotic intervention may reduce bilirubin levels without affecting other hematologic parameters.

Discussion

The present study demonstrates that Pedilact, when used as an adjunct to phototherapy, significantly lowers bilirubin levels in low birth weight (LBW) infants with jaundice, particularly at 24, 48, and 72 hours post-treatment. These results are consistent with previous research, such as Kamel et al. (2019), who observed a notable reduction in bilirubin levels in term infants receiving probiotics alongside phototherapy (7). Similarly, Mutlu et al. (2018) reported that *Lactobacillus rhamnosus* GG decreased bilirubin levels in term neonates by 36 hours (8). The current study extends these observations to LBW infants, a population at heightened risk of severe hyperbilirubinemia due to immature physiological systems (2).

The potential mechanisms by which probiotics reduce bilirubin include modulation of gut microbiota, enhanced intestinal motility, and decreased enterohepatic circulation. *Lactobacillus* and *Bifidobacterium* species may reduce β-glucuronidase activity, limiting the deconjugation and reabsorption of bilirubin in the gut (5,10). Moreover, probiotics may accelerate the passage of meconium, facilitating bilirubin excretion (6). These mechanisms are particularly relevant in LBW infants, who often exhibit delayed gut colonization and impaired bilirubin metabolism (11).

Comparable studies support these findings. Saeidi et al. (2016) reported that probiotic supplementation in preterm infants significantly

reduced both bilirubin levels and phototherapy duration (12). Farhat et al. (2025) found that a five-day course of probiotics effectively lowered bilirubin in preterm neonates without prior phototherapy, while in those previously treated, a significant reduction was observed on day six. No significant effect on weight gain was detected, confirming the safety of probiotics as an adjunct therapy in neonatal jaundice (13).

These results are further corroborated by Armanian et al. (2016), who demonstrated improved feeding tolerance and reduced bilirubin levels in preterm infants (<1500 g) receiving prebiotic supplementation, highlighting the role of gut microbiota modulation in jaundice management (14).

However, some studies report inconsistent outcomes. Zahed Pasha et al. (2017) observed no significant difference in phototherapy duration with probiotic use in term infants, suggesting that efficacy may depend on probiotic strains, dosage, or patient characteristics (15). Variations in study populations (term vs. preterm) and probiotic formulations may account for these discrepancies. For example, our study utilized Pedilact, a multi-strain probiotic, which may exert synergistic effects compared to single-strain formulations (16).

The clinical implications of these findings are substantial. A faster reduction in bilirubin levels may shorten hospital stays, decrease healthcare costs, and lessen the emotional and financial burden on families. Moreover, probiotics such as Pedilact are cost-effective and widely accessible, supporting their use as an adjunct therapy. The results demonstrated significant efficacy at 24, 48, and 72 hours, with the magnitude of improvement becoming more pronounced over time, likely due to the period required for effective gut colonization by the probiotic (14).

Limitations

Limitations of this study include the relatively short follow-up period (72 hours), which restricts the evaluation of long-term outcomes such as neurodevelopmental status or potential late adverse effects. Furthermore, the study focused exclusively on preterm LBW infants without hemolytic disease, limiting generalizability to term infants or those with hemolytic jaundice. The retrospective trial registration is another limitation that should be acknowledged. Strengths of the study include its double-blind design, adequate sample size, standardized probiotic formulation, and detailed blinding procedures, enhancing the reliability of the results.

Future research should examine the long-term safety and effectiveness of probiotics in neonatal jaundice, including potential impacts on neurodevelopment. Comparative studies evaluating different probiotic strains and dosages could optimize treatment protocols. Cost-effectiveness analyses, as recommended by Saeidi et al. (2018), may further elucidate the economic benefits of probiotic supplementation in resource-limited settings (17).

Conclusion

Pedilact supplementation as an adjunct to phototherapy effectively enhances bilirubin decline in preterm low-birth-weight infants with jaundice, contributing to shorter hospital stays. Its cost-effectiveness and availability make it a practical and beneficial addition to current neonatal jaundice management.

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Conflicts of interest

The authors declare no conflicts of interest.

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