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Original Article

Effects of Probiotics on Serum Bilirubin Levels in Low –Birth-Weight Infants

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ABSTRACT

Background: Neonatal jaundice is frequently observed in preterm neonates, necessitating significant medical interventions, including phototherapy. This investigation assesses the impact of probiotics on serum bilirubin levels in preterm neonates with weights ranging from 1000 to 2500 grams.

Methods: In this single-blind randomized clinical trial conducted at Imam Reza Hospital (Mashhad, Iran) during 2019-2020 80 Low- Birth weight (LBW) neonates (1000-2500 g) were enrolled. The intervention group received 5 drops of probiotic daily for 5 consecutive days, initiated within 48 hours of birth. Transcutaneous bilirubin was measured daily for 7 days; serum bilirubin was assessed when transcutaneous values exceeded thresholds.

Results: The trial comprised 40 neonates in the probiotic group and 40 in the control group. Statistical analysis revealed no significant baseline characteristic differences between the groups. Notably, in the subset of neonates without prior phototherapy, those administered showed a statistically significant reduction in bilirubin levels compared to controls (p value< 0.05). However, among neonates previously subjected to phototherapy, bilirubin levels, only on the sixth day, were statistically different between the probiotics and control groups (p value= 0.002) and bilirubin reduction did not significantly vary in the other days.Weight gain during the first week of the study, did not show a significant difference between two groups (p values were 0.392 and 0.632 in neonates with and without phototherapy, respectively).

Conclusion: Probiotics, may effectively reduce serum bilirubin levels in preterm neonates, particularly those who have not undergone phototherapy.

Keywords: Hyperbilirubinemia, Low- Birth weight, Neonate, Probiotics

Introduction

Neonatal jaundice, characterized by elevated serum bilirubin, is common, affecting around 60% of term and 80% of preterm neonates (1). While often physiological, it can be pathological, leading to complications like kernicterus if untreated (2). Physiological jaundice typically resolves within two weeks, but high bilirubin levels in some neonates require intervention. Pathological jaundice is defined by early onset, rapid bilirubin increase, or high bilirubin levels (3,4).

Currently, several treatment methods have been proposed for neonatal jaundice, such as fluid therapy, infant ear cupping, melatonin administration, probiotics, phenobarbital, and more (5-8).

Probiotics are explored as adjunctive therapy, modulating gut microbiota to enhance bilirubin excretion (11). Recent studies suggest probiotics combined with phototherapy may shorten phototherapy duration, lower rebound hyperbili-

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rubinemia, and reduce hospital stays (12). However, not all studies show a benefit. Some studies indicate potential benefits of specific probiotics in VLBW neonates and healthy neonates, while others highlight the potential of prebiotics in preterm infants (13, 14). One study found no significant reduction in hospital stay with probiotic use (15).

This study aims to evaluate the efficacy and safety of probiotics in reducing serum bilirubin levels in preterm neonates weighing 1000-2500 grams. This research seeks to offer an alternative to conventional treatments for this vulnerable population.

Methods

This study employed a single-blind, randomized controlled clinical trial design. The research was conducted at the Neonatal Intensive Care Unit (NICU) of Imam Reza Hospital in Mashhad, Iran, over a one-year period spanning from 2019 to 2020.

The study population consisted of 80 neonates with birth weights between 1000 and 2500 grams. Inclusion criteria were: (a) absence of significant congenital anomalies; (b) no history of mechanical ventilation or intubation; (c) absence of heart failure or severe asphyxia; and (d) no Rh incompatibility or positive Coombs test. Exclusion criteria included the development of conditions requiring blood exchange transfusion during the study period. These criteria were established to ensure a homogenous study group and to minimize potential confounding factors that could influence serum bilirubin levels.

Following informed consent, eligible neonates were randomly assigned to either the intervention group or the control group using a block randomization method. This method ensured an equal number of participants in each group. Due to practical limitations, a single-blind design was implemented, where outcome assessors and data analysts were blinded to the group allocation. Parents and clinical staff, however, were aware of the treatment assignments.

This study used Pedilact drops, which are a special probiotic combination that contains high amounts of 3 strains of beneficial bacteria including Lactobacillus reuteri, Lactobacillus rhamnosus, Bifidobacterium infantis, and the prebiotic fructooligosaccharide, which can be consumed by infants. The intervention group received 5 drops of Pedilact daily for five consecutive days. The control group received no probiotic supplementation. The intervention was designed to evaluate the effect of probiotic supplementation on bilirubin metabolism and excretion in neonates.

Transcutaneous bilirubin (TcB) measurements were obtained daily for each neonate over a seven-day period to monitor bilirubin levels. If TcB levels exceeded the established normal range for the neonate's age, a serum bilirubin measurement was performed for accurate assessment.

The primary outcome measure was the difference in serum bilirubin levels between the intervention and control groups. Secondary outcome measures included the need for phototherapy and the occurrence of any adverse effects potentially related to probiotic administration.

Data were analyzed using SPSS version 16.0. Descriptive statistics, including means and standard deviations, were calculated for continuous variables, and frequencies were used to summarize categorical variables. Independent t-tests were used to compare continuous variables between the two groups. Repeated measures ANOVA was employed to analyze the changes in bilirubin levels over time within and between the groups. A p-value of less than 0.05 was considered statistically significant.

Ethical Approval

The protocol was approved by the Ethics Committee of Mashhad University of Medical Science (Approval code: IR.MUMS.MEDICAL. REC.1399.576).

Written informed consent was obtained from the parents or legal guardians of all participating neonates prior to enrollment.

Results

The study included 80 neonates, equally divided into the probiotic (n = 40) and control (n = 40) groups. The sample comprised 49 male (61.25%) and 31 female (38.75%) neonates. Baseline demographic and clinical characteristics, including gestational age, birth weight, and previous health history, were comparable between the two groups, ensuring baseline equivalence for assessing the intervention's impact. Analysis of serum bilirubin levels revealed a statistically significant decrease in the probiotic group compared to the control group among neonates without prior phototherapy exposure ($p < 0.05$) (Table 1).

This suggests that probiotics may have a beneficial effect on reducing bilirubin levels in preterm neonates who have not previously

Table 1. Comparison of bilirubin levels between two probiotic groups in infants who did not receive phototherapy

Bilirubin level (mg/dl)	probiotic		p-value**
	no	yes	
Bilirubin day1	8.42±1.18	8.66±1.84	0.575
Bilirubin day2	10.33±2.12	9.53±1.81	0.154
Bilirubin day3	11.5±2.15	9.27±1.76	0.000
Bilirubin day4	11.46±2.17	8.98±1.65	0.000
Bilirubin day5	11.58±2.43	8.82±1.81	0.000
Bilirubin day6	11.29±2.39	9.12±1.98	0.002
Bilirubin day7	10.88±2.05	8.55±2.01	0.050
p-value*	0.006		

repeated measure ANOVA*
T-test**

undergone phototherapy. In contrast, among neonates who had undergone phototherapy, the differences in bilirubin levels between the probiotic and control groups were not statistically significant, with the exception of day six. On day six, bilirubin levels were higher in the probiotic group. This could suggest that the bilirubin level increases later in the serum of low-weight neonates. Additionally, it could suggest that the effect of probiotics on bilirubin reduction may be influenced by prior phototherapy treatment (Table 2).

The study monitored weight gain in neonates across both groups. While the probiotic group exhibited a trend towards higher weight gain, the difference was not statistically significant compared to the control group ($p > 0.05$).

Table 2. Comparison of bilirubin levels between two probiotic groups in infants who underwent phototherapy

Bilirubin level (mg/dl)	probiotic		**p-value
	no	yes	
Bilirubin day1	11.31±1.85	10.72±2.05	0.414
Bilirubin day2	11.94±2.21	10.89±2.65	0.245
Bilirubin day3	11.69±2.75	10.07±1.91	0.076
Bilirubin day4	11.13±2.78	9.75±1.67	0.118
Bilirubin day5	11.00±2.58	9.71±2.38	0.167
Bilirubin day6	12.13±2.73	9.17±1.93	0.002
Bilirubin day7	11.06±2.46	9.33±2.12	0.050
p-value*	0.655		

repeated measure ANOVA*
**T-test

Discussion

The primary aim of this study was to evaluate the efficacy of probiotic supplementation in reducing serum bilirubin levels in preterm neonates with low birth weight. Our findings indicate that probiotic administration significantly decreased bilirubin levels in neonates who had not previously undergone phototherapy, suggesting a potential beneficial role of probiotics in managing neonatal hyperbilirubinemia in this vulnerable group.

The significant reduction in serum bilirubin among neonates who had not received

phototherapy aligns with previous studies suggesting that probiotics may enhance bilirubin clearance. The proposed mechanism involves modulation of the gut microbiota, leading to increased deconjugation and excretion of bilirubin. By restoring or promoting a healthy balance of intestinal bacteria, probiotics might reduce enterohepatic circulation of bilirubin, thereby facilitating its elimination (10, 11). Our results support these hypotheses, indicating that probiotics could serve as a non-invasive adjunct or alternative in managing hyperbilirubinemia, especially in cases where phototherapy may not be immediately indicated or when seeking to reduce phototherapy duration.

However, in neonates previously treated with phototherapy, the differences between probiotic and control groups were not statistically significant, and interestingly, bilirubin levels increased slightly in the probiotic group on day six. This may suggest that prior phototherapy alters the gut environment or bilirubin metabolism in such a way that probiotics have limited or delayed effects. Alternatively, the effect of phototherapy itself on gut microbiota or hepatic bilirubin conjugation pathways may confound the efficacy of probiotics. It is also plausible that these neonates have a different bilirubin kinetic profile or underlying pathology that diminishes probiotic effectiveness.

The pathophysiology of neonatal hyperbilirubinemia is complex, involving increased bilirubin production, immature hepatic conjugation, and intestinal elimination. Probiotics are believed to improve bilirubin elimination mainly through:

- Enhancing gut microbiota diversity and activity, particularly of bacteria capable of converting conjugated bilirubin to less reabsorptive forms.
- Producing enzymes such as β-glucuronidase, which can influence bilirubin metabolism.
- Strengthening gut barrier integrity, reducing enterohepatic recirculation.
- Modulating systemic inflammation, indirectly supporting hepatic function.

These mechanisms are supported by animal and human studies indicating that probiotic supplementation can favorably alter bilirubin metabolism (10, 12). Nonetheless, the specific strains of probiotics, timing, and dosage are critical factors that can influence outcomes.

Although probiotics showed a trend toward improved weight gain, the differences were not statistically significant. This aligns with some existing literature suggesting that short-term probiotic administration may not markedly influence short-term growth in preterm infants (14, 15). Longer-term studies may be required to detect potential benefits on growth parameters, as well as other health outcomes, such as immune function and gastrointestinal health.

Despite promising results, this study has several limitations. The sample size, while adequate for detecting certain differences, may limit the generalizability of findings. The single-blind design, although pragmatic, can introduce bias. Additionally, the study focused solely on immediate bilirubin reduction without long-term follow-up, which may be necessary to determine sustained benefits.

Future research should consider larger, multicenter trials with multiple probiotic strains, varied dosages, and longer follow-up periods. Investigations into the mechanistic pathways through microbiome profiling and hepatic function assessments could elucidate how probiotics influence bilirubin metabolism. Moreover, evaluating the interplay between probiotics and other treatments, such as phototherapy or pharmacological agents, could offer insights into integrated management strategies.

Conclusion

The results of our study indicate that probiotics, may effectively reduce serum bilirubin levels in preterm neonates, particularly those who have not undergone phototherapy.

Acknowledgments

None.

Conflicts of interest

None.

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