Original Article

Effectiveness of Probiotics and Phototherapy Compared to Phototherapy Alone in Treating Hyperbilirubinemia in Neonates ≥35 Weeks Gestation: A Comparative Observational Study

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Abstract

Introduction: Neonatal jaundice is a common condition in neonatal practice. Phototherapy is accepted first line of therapy in its treatment. Probiotics can be an addition as it decreases enterohepatic circulation and improves gut function.

Materials and Methods: The observational study was conducted in 40 neonates. Participants were divided into 2 groups, phototherapy alone (PA) and probiotics plus phototherapy (PP). The mean difference in total serum bilirubin (mg/dL) levels, total duration of phototherapy, incidence of rebound hyperbilirubinemia, frequency of stooling after initiation of therapy, feed intolerance, and occurrence of new onset sepsis was compared.

Results: The mean difference in serum bilirubin level was not statistically significant after 12 hours (p = .321), 24 hours (p = .544) and 48 hours (p = .863) after initiation of phototherapy. An increase in frequency of stool in the first 24 hours was observed in PP group (p = .003). The total duration of phototherapy was less in PP group although not statistically significant (25 hours vs 38 hours, p = .054). Rebound hyperbilirubinemia was significantly higher in PA (p = .023). There were no cases of feed intolerance and sepsis.

Conclusion: The addition of probiotics did not alter the clinical course of jaundice. However, probiotics can reduce the rebound hyperbilirubinemia and is safe as it did not increase incidence of new onset sepsis or feed intolerance.

Keywords

Birth order, birth weight, neonatal and infant nursing, neonatology/perinatology, newborn

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Introduction

Neonatal jaundice is a common condition encountered in clinical neonatology practice.[1] Yellowish discoloration of skin and sclera is caused by deposits of excessive bilirubin.[2] Neonatal hyperbilirubinemia occurs in about 60% of term and 80% of preterm neonates.[3]

In neonates, decreased bowel frequency and stasis of intestinal contents lead to enterohepatic recirculation and thus increase serum bilirubin levels.[1] In addition, the gut microbiota profile shows an increase in the proliferation of pathogenic organisms and a relative deficiency in nonpathogenic bacteria like Bifidobacterium, thus contributing to increased bilirubin levels.[4] Phototherapy is a widely accepted first line of therapy in the treatment of neonatal jaundice, but it may cause several short- and long-term side effects like DNA damage, which possibly can be prevented by shortening the duration of phototherapy by the addition of measures to enhance bowel clearance such as probiotics.[5] Probiotics decrease enterohepatic circulation and improve gut functions thus helping in decreasing serum bilirubin levels,[6] also supplementation of probiotics decreases jaundice by regulating colonies and enhancing immunity.[7]

Prolonged stay in NICU due to jaundice causes a financial burden to families and leads to the separation of mother and baby which is emotionally disturbing for the mother and not a developmentally supportive practice for the neonate as it leads to mother and baby separation.[8]

It was hypothesized that giving probiotics to the neonates is likely to improve the effectiveness of phototherapy with regard to bilirubin reduction rate and duration of treatment among the neonates of ≥35 weeks gestation with

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unconjugated hyperbilirubinemia. This is likely to decrease the duration of NICU stay and mother-baby separation, create greater mother-baby bonding, encourage breastfeeding, and even mitigate the side effect of phototherapy by decreasing its total duration.

Material and Methods

The comparative interventional study was carried out in level III NICU and mother and neonatal unit (MANU) of the Department of Neonatology at a tertiary care setup in Uttarakhand. The time period of study was 4 months (December 2021 to March 2022). Written and informed consent was obtained from parents or guardians of eligible neonates, and institutional ethical clearance was taken prior to enrolling subjects.

Primary Objective

To compare the effectiveness of probiotics and phototherapy (PP) with phototherapy alone (PA) by measuring the mean difference in total serum bilirubin (TsB, mg/dL) levels in neonates with unconjugated hyperbilirubinemia.

Secondary Objective

To determine the total duration of phototherapy, incidence of rebound hyperbilirubinemia, frequency of stooling after initiation of therapy, need for IVIg, need of double volume exchange transfusion (DVET), incidence of new-onset sepsis (defined by positive blood culture or 2 positive sepsis screen parameters), and incidence of feed intolerance (as decided by the treating consultant) among the groups.

Inclusion Criteria

Neonates with a gestational age of 35 weeks or more with unconjugated hyperbilirubinemia due to any cause, requiring phototherapy as per the American Academy of Paediatrics charts were included.

Exclusion Criteria

Neonates kept nil by mouth for any reason, neonates receiving phenobarbitone/ursodeoxycholic acid/undergone exchange transfusion (DVET) prior to admission, or if surgical cause was suspected were excluded.

Methodology

The eligible neonates were divided into 2 groups—group PA (phototherapy alone) and group PP (phototherapy plus probiotics) using a computer-generated number sequence. Neonates in group PA received only phototherapy as per existing NICU protocol, and neonates in group PP received phototherapy along with probiotics (Darolac sachet, Aristo Pharmaceuticals Pvt Ltd, containing Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum and Saccharomyces boulardii 1.25 billion cells per gram of sachet). One sachet was dissolved in 5 ml of expressed breast milk/formula feed and administered twice daily for 3 consecutive days.[9,10] Neonates in both groups received intensive phototherapy. Phototherapy was administered continuously using the Zeal LED phototherapy unit except during periods of diaper change, feeding, physical examination, and blood sampling. The intensity of phototherapy at the level of skin was checked at the initiation and every 12 hours, by using a fluxmeter (Fanem/Thor multitester MOD-3620) and was kept > 30 $\mu W/cm^2/nm$, throughout the duration of therapy. The neonates were nursed in an open care system wearing only an eye patch and a diaper which was kept folded to allow maximum skin exposure to phototherapy.

TsB level was evaluated every 12 hours or earlier as decided by the treating physician till phototherapy was continued. Rebound TsB was done at 12 hours after stopping phototherapy.

Neonates were closely monitored for any short-term side effects of probiotics including vomiting, feed intolerance, abdominal distension, loose stool, necrotising enterocolitis (NEC), and sepsis. Phototherapy was stopped when serum bilirubin levels were below 2 mg/dL of the phototherapy range.

Sample Size

A total of 64 neonates (32 in each group with 1:1 allocation) were planned to be studied. The sample size was calculated based on the difference in serum bilirubin levels between the group receiving probiotics with phototherapy 10.83 ± 1.54 and PA 11.91 ± 1.54 at 36 hours of starting phototherapy based on a previous study, with α 0.05 and power of 80%.[6] But due to the rise of COVID-19 cases during the study period, fewer neonates could be enrolled as it was a timed research project for academic needs.

Statistical Analysis

Data were entered in an excel spreadsheet, cleaned, and coded. The presentation of the categorical variables was done in the form of numbers and percentages (%), and the quantitative data were presented as the mean ±SD and as median with 25th and 75th percentiles (interquartile range). The normality of data was checked by using the Kolmogorov-Smirnov test. The comparison of quantitative variables with non-normal distribution was done by Mann-Whitney Test and Independent t-test to compare normally distributed data between two groups. The comparison of the qualitative variables was done using the chi-square test or Fisher's exact Test. Statistical Package for Social Sciences (SPSS) software, version 21.0.

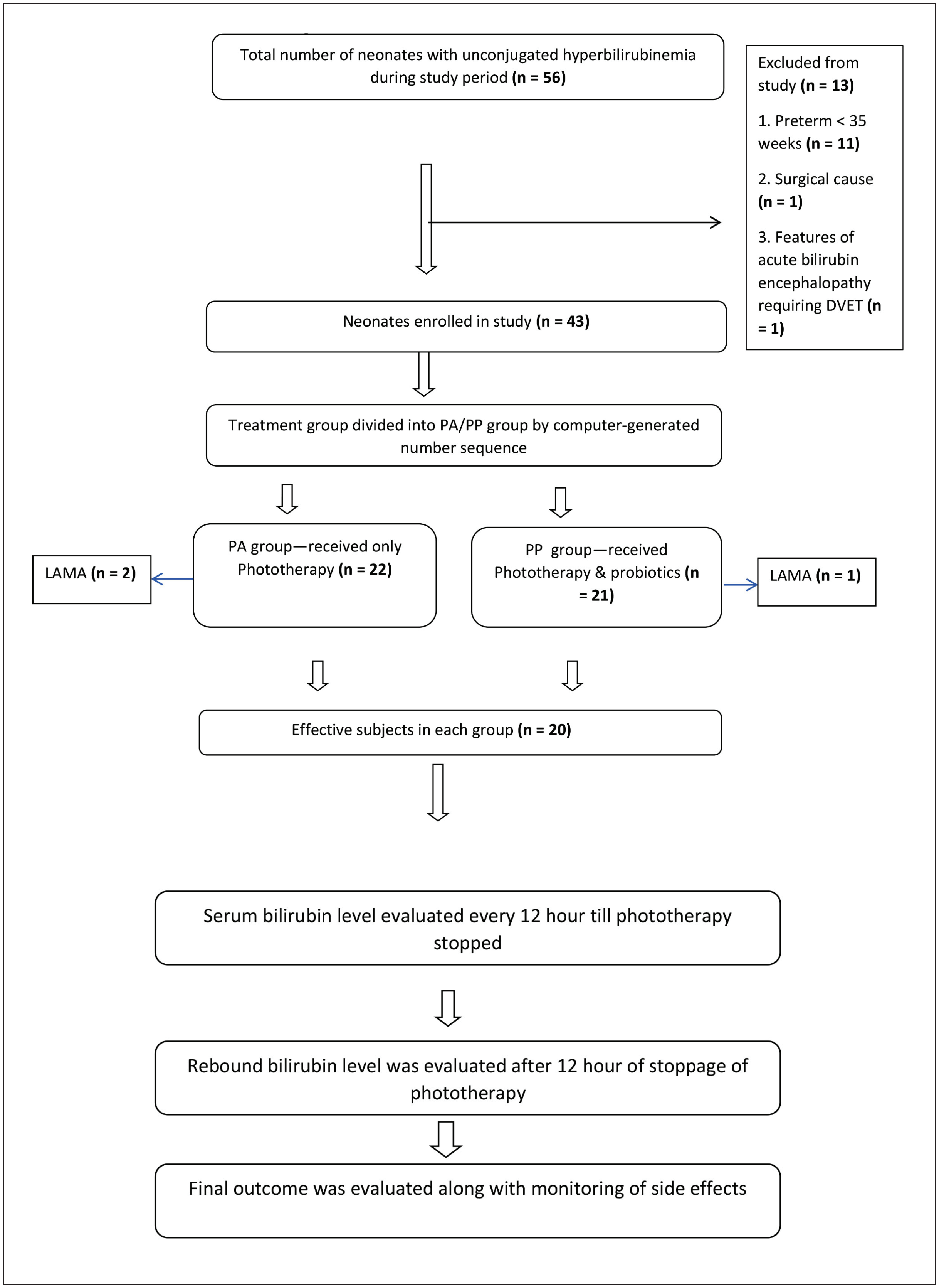

Figure 1. Study Flow Chart.

Table 1. Demographic Parameters of Study Population.

Parameters	PA Group	PP Group	p Value
Place of birth	17 (85%)	15 (75%)	.695
Inborn, N (%)			
Gender	12 (60%)	11 (55%)	.749
Male, N (%)			
Birth weight (gm)	2669 ± 349	2790 ± 538	.524
Mean±SD			
Gestational age (weeks)	38.25 ± 0.60	38.66 ± 0.32	.325
Mean±SD			
Age at start of phototherapy in hours of life, N (%)			
<24 hours	0 (0%)	1 (5%)	.961
25–48 hours	7 (35%)	9 (45%)	
49–72 hours	4 (20%)	3 (15%)	
73–96 hours	3 (15%)	2 (10%)	
97–120 hours	3 (15%)	2 (10%)	
≥121 hours	3 (15%)	3 (15%)	

(IBM manufacturer, Chicago, USA), was used for data analysis. For statistical significance, a p value of less than .05 was considered statistically significant.

Results and Observation

Study Flow Chart

Figure 1 shows the study flow chart. A total of 56 neonates presented with hyperbilirubinemia during the study period, out of which 13 were excluded. 43 neonates were included and allocated in two groups (PA=22 and PP=21).

Results

A total of 40 neonates were enrolled for analysis, 20 in each group. Demographic characteristics of enrolled neonates are depicted in Table 1. A total of 80% of enrolled neonates were inborn with a male-to-female ratio was 1.35:1. Mean birth weight was 2669 ± 349 grams in the PA group, and 2790 ± 538 grams in the PP group and the mean gestational age of the neonates enrolled in the study was 38.25 ± 0.60 weeks in PA group and 38.66 ± 0.32 weeks in PP group, which were similar. Phototherapy was started between 25 and 48 hours of life in the majority of the neonates, in both groups. Both groups were comparable in baseline parameters.

Figure 2 depicts the predominant causes of jaundice in the study population. In the majority 10 (25%) of patients, the cause of neonatal hyperbilirubinemia was breastfeeding jaundice followed by late prematurity 9 (22.50%), ABO setting 8 (20%), ABO incompatibility 6 (15%), Rh setting 4 (10%), Rh incompatibility 2 (5%), and minor blood group incompatibility 1 (2.50%).

The primary outcome of the study was the mean difference in serum bilirubin level which was not statistically significant after 12 hours (p = .321), 24 hours (p = .544), and 48 hours (p = .863) after initiation of phototherapy (Table 2).

The secondary outcomes were compared in Table 3. The mean ± SD of rebound TsB (mg/dL) after 12 hours of stoppage of phototherapy in the PA group was 10.3 ± 2.49 which was significantly higher than the PP group 8.6 ± 2.01(p = .023). The total duration of phototherapy was less in babies receiving PP compared to PA although not statistically significant (25 hours vs 38 hours, p = .054). There was no significant difference in the occurrence of rebound hyperbilirubinemia requiring re-inititaion of phototherapy. A significant increase in stool frequency in the first 24 hours was observed in the PP group (p = .003). However, this effect was not observed at 24 to 48 hours after

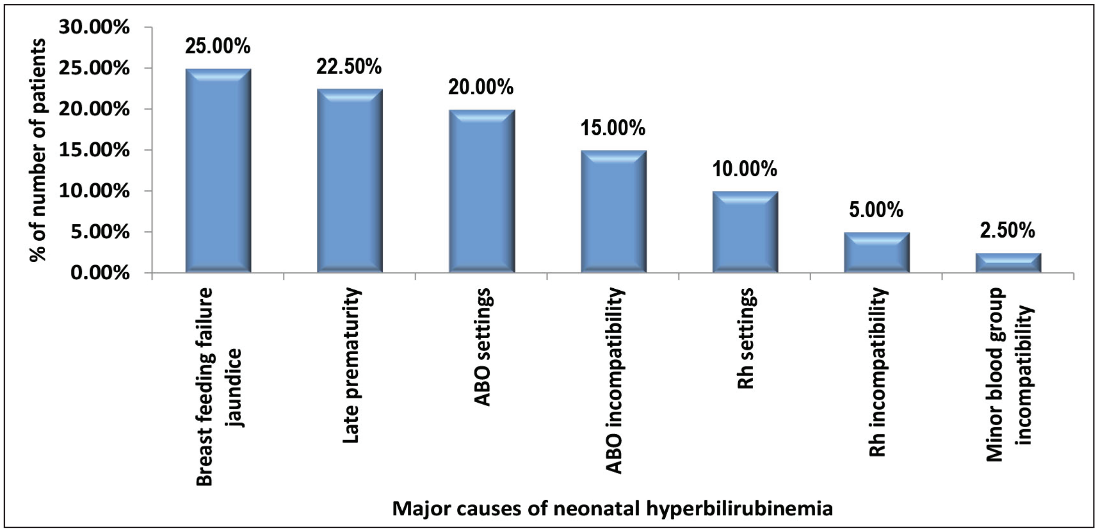

Figure 2. Distribution of Major Causes of Neonatal Hyperbilirubinemia of Study Subjects.

Table 2. Comparison of Mean Difference in Total Serum Bilirubin (mg/dL) in the 2 Groups.

Duration of Phototherapy	PA Group Mean ± SD	PP Group Mean ± SD	*p* Value
After 12 hours	0.88 ± 1.49	1.37 ± 1.57	.321
After 24 hours	1.3 ± 1.84	1.6 ± 1.04	.544
After 48 hours	–0.2 ± 0.94	–0.38 ± 0	.863

Table 3. Comparison of Secondary Outcomes in the 2 Groups.

	PA (*n* = 20)	PP (*n* = 20)	*p* Value
Rebound TsB (mg/dL) 12 hours after stopping phototherapy Mean ± SD	10.3 ± 2.49	8.6 ± 2.01	**.023**
Total duration of phototherapy (hours) Median (25th-75th percentile)	38 (27–51)	25 (24–36)	.054
Number of neonates required repeat phototherapy for rebound hyperbilirubinemia n (%)	4 (20%)	0 (0%)	.106
Frequency of stool			
In first 24 hour Mean ± SD	4.2 ± 1.2	5.6 ± 1.43	**.003**
In next 24 to 48 hours of phototherapy Mean ± SD	4.8 ± 1.85	5.15 ± 1.6	.516
In 48 to 72 hours of phototherapy Mean ± SD	4.6 ± 1.57	4.85 ± 1.23	.345

Note: Bold values indicate significant *p* values.

phototherapy (p = .516) and after 48 to 72 hours of phototherapy (p = .345). There was no incidence of new-onset sepsis, feed intolerance, and no need for IVIg and DVET in any of the enrolled babies in both groups.

Discussion

Out of 40 enrolled babies, 80% were inborn, predominantly male neonates. The mean TsB level at the start of phototherapy was similar in both groups; 14.40 ± 3.44 mg/dL in group PA and 14.15 ± 3.25 mg/dL in group PP. Phototherapy was initiated at 25 to 48 hours of life in most babies.

The mean difference in serum bilirubin level (mg/dL) after 12, 24, and 48 hours of phototherapy was 0.88 ± 1.49, 1.3 ± 1.84, –0.2 ± 0.94 respectively in the PA group and 1.37 ± 1.57, 1.6 ± 1.04, –0.38 ± 0, respectively, in PP group, which is similar to study done by Liu Wet al.[11] Although the mean difference in serum bilirubin level (mg/dL) was more in the probiotics PP group, it was not statistically significant which could be due to a small sample size.

The incidence of rebound hyperbilirubinemia was higher in the group receiving only phototherapy than in the phototherapy plus probiotics group. This finding is similar to that reported by Habibi et al.[12] Probiotics help better control neonatal jaundice by improving enterohepatic circulation and thus reduce the chance of increased rebound bilirubin.

The mean duration of phototherapy in group PP was shorter than the PA group, which is similar to the study done by Demirel G et al.[13] Chen et al have also reported that if phototherapy is supplemented with probiotics then the duration of phototherapy is decreased and it translates to a lesser duration of hospital stay. Probiotics improve enterohepatic circulation and thus reduce the duration of phototherapy. This was confirmed in a meta-analysis done by Deshmukh et al. He included 2040 neonates in his meta-analysis and found that probiotic supplementation reduces the duration of phototherapy in infants with jaundice.[8]

Stool frequency in our study was significantly higher in groups receiving phototherapy plus probiotics compared to PA (p < .5) during the initial 24 hours of therapy, whereas subsequently there is no difference between the groups, which is similar to the study done by Ashiana et al.[14]

None of the babies in either of the groups developed any adverse effects of probiotics during the study period which is similar to other studies.[7,11]

The limitations of our study remain the observational nature of the study and the small sample. A study including preterm of all gestational age will give further well-defined answers.

Conclusion

The addition of probiotics to phototherapy did not lead to a significant difference in the mean difference in serum bilirubin level after initiation of phototherapy. However, probiotics can reduce the rebound hyperbilirubinemia after stoppage of phototherapy. Probiotic usage did not lead to increase in incidence of new onset sepsis or feed intolerance.

Author Contributions

SS and GG conceptualized and planned the study. RK, SS, SW did the acquisition of data, analysis, and interpretation of results, literature review, and drafted the initial manuscript. SP and CC edited and drafted the final manuscript. GG critically reviewed the manuscript and would act as guarantor of the paper.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Ethical approval from Institutional Ethical Committee was obtained.

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Informed Consent

The participant has consented to the submission of the article to the journal.

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