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Original Article

Probiotic Use and Unconjugated Hyperbilirubinemia in Neonates: A Prospective Cohort Study in Sulaimani, Kurdistan region/Iraq

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Abstract

Background: Neonatal unconjugated hyperbilirubinemia (UHB) is common and may require phototherapy (PT). This study evaluated the effect of probiotic supplementation, as an adjunct to PT, on total serum bilirubin (TSB) kinetics in neonates with UHB in Sulaimani, Kurdistan Region/Iraq.

Method: In this prospective interventional cohort (January 2024–December 2025), 156 neonates (≥34 weeks, ≤10 days old) with predominantly UHB were allocated into four groups: PT + probiotic (n=24), PT + no probiotic (n=24), no PT + probiotic (n=70), and no PT + no probiotic (n=38). A standardized Lactobacillus preparation was used. TSB and packed cell volume (PCV) were measured on days 1, 2, 3, 5, and 7.

Result: Initial mean TSB was higher in PT groups (14.88 ± 1.41; 14.81 ± 1.25 mg/dL) than in no-PT groups (12.20 ± 0.75; 12.60 ± 0.70 mg/dL; p<0.001). Probiotics had no significant impact on early TSB decline overall (rate difference 0.06 mg/dL/day; 95% CI –0.08 to 0.20; p=0.385). During days 4–7, probiotics significantly increased TSB decline overall (0.32 mg/dL/day; 95% CI 0.18–0.46; p<0.001), particularly in neonates without PT (0.38 mg/dL/day; 95% CI 0.25–0.51; p<0.001), and reduced time to TSB <12 mg/dL (2.8 ± 0.6 vs. 4.1 ± 0.9 days; p<0.001). No benefit was observed among infants receiving PT.

Conclusion: In late preterm and term neonates with mild to moderate UHB managed without PT, probiotics significantly accelerate late-phase bilirubin decline and shorten time to safe TSB levels.

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1. Introduction:

Neonatal jaundice, clinically recognized by a yellowish discoloration of the skin, sclera, and mucous membranes, is among the most common clinical conditions encountered in newborns (1). Hyperbilirubinemia is caused due to deposition of bilirubin in the blood. Nearly 60% of term and up to 80% of preterm infants have this condition during the first week of living, with most cases being benign physiologic jaundice (2). If left unchecked, pathological hyperbilirubinemia has the potential to develop into serious complications, including bilirubin-induced neurologic dysfunction (BIND) and kernicterus (3).

Neonatal hyperbilirubinemia is a multifactorial disorder caused by increased production of bilirubin, immature hepatic (liver) conjugation processes, and enhanced enterohepatic circulation (4). Because of their high turnover

of red blood cells, immature uridine diphosphate glucuronosyltransferase (UGT) enzyme systems, and delayed establishment of gut microbiota, neonates are particularly susceptible to this condition (5). Bacterial β-glucuronidase can deconjugate bilirubin in the intestines, allowing for the reabsorption of unconjugated bilirubin (UB) into the bloodstream, thereby continuing to elevate serum bilirubin levels (6). This process is increasing in neonates due to an increased amount of the enzyme and a lack of colonization by protective bacteria (7).

Phototherapy (PT) and exchange transfusion are conventional management approaches. When a newborn has severe jaundice, PT is usually successful in converting bilirubin into water soluble photoisomers. However, due to its effectiveness and the disruptive effect on mother-child bonding and other risks (dehydration, hypocalcemia, and possibly long-term implications); PT has generated concern (8, 9). On the other hand, although exchange transfusion may save the life of the baby, it is an invasive procedure and could lead to a number of negative outcomes including: arrhythmias, electrolyte imbalance, and infection

(2). Therefore, there has been an increased interest in safe complementary medical interventions.

Probiotics are defined as viable microorganisms that provide health benefits to the host when given in sufficient amounts (10). Probiotics have been proposed as a potential treatment for lowering bilirubin in various ways, including modifying the gut microbiota; inhibiting β-glucuronidase activity; stimulating gastrointestinal motility; lowering intestinal pH; and increasing the formation of nonabsorbed bilirubin metabolites (11, 12).

Multiple clinical studies provide supporting evidence for the efficacy of probiotics as adjunct therapy. For instance, Rezki et al. (2023) demonstrated how Lactobacillus reuteri, when administered in conjunction with PT, markedly expedited the reduction of and indirect bilirubin levels as compared to PT treatment alone ($p<0.001$) (13). Likewise, Tariq et al. (2021) also reported that probiotics appear to shorten duration of PT treatment for neonates with hyperbilirubinemia, without producing negative outcomes ($p=0.003$) (14). Collectively, these studies suggest that probiotics may play an adjunctive role in treating neonatal jaundice.

Researchers have yet to reach consensus despite promising findings from prior studies. While certain studies observed a statistically significant drop in bilirubin, other studies documented minimal to nil effect (15, 16). Also, in studies mentioning a probiotic effect, the study population was predominantly Western or not specified in their ethnic origin, allowing for potential discrepancies from other populations. Importantly, no research has evaluated the effect of probiotics on neonatal hyperbilirubinemia in the Kurdistan Region of Iraq, an environment with its own specific demographics, genetics, and healthcare system. For this reason, the study aimed to assess if probiotics have an effect on serum UB levels in neonatal hyperbilirubinemia receiving PT in Sulaimani, Kurdistan Region, Iraq.

2.Materials and Methods:

2.1Study Design and Setting

This prospective interventional cohort study was conducted in Sulaimani, Kurdistan Region of Iraq, between January 2024 and December 2025. The study took place in two tertiary care centers providing level III neonatal intensive care services: the Neonatal Intensive Care Unit (NICU) of Sulaimani Maternity Teaching Hospital and the NICU and neonatal wards of Dr. Jamal Ahmed Rasheed Pediatric Teaching Hospital. And 2 out- patient private clinics of the authors.

2.2 Participants

Total of 156 neonates were enrolled in this study. 94 of them received probiotics, while 62 of them did not receive it. Participants were

late preterm and term neonates presenting with unconjugated hyperbilirubinemia (UHB), either admitted for PT or managed as outpatients.

Eligible infants were identified by the treating neonatologists during routine clinical care. Parents or legal guardians of potentially eligible neonates were approached by a research nurse, provided with a standardized information sheet, and invited to participate. Recruitment continued until the required sample size was reached. After written informed consent was obtained, enrolled infants were allocated to one of four analytic groups based on two factors determined by routine clinical decisions: need for PT (yes/no) and receipt of probiotic supplementation (yes/no).

2.3 Inclusion criteria:

Neonates were enrolled if they had a minimum of 34 completed weeks of gestation when the baby was born, were less than 14 days old from birth when enrolled and had evidence from lab work and clinical signs indicating that primarily they had UHB which required inpatient PT or structured outpatient follow-up, per the institution's guidelines. Only infants whose parents or legal guardians provided written informed consent were enrolled.

2.4 Exclusion criteria:

neonates were excluded if they were extremely premature or had very low birth weight; they had major congenital anomalies affecting survival, feeding, or bilirubin metabolism; clinical or laboratory evidence of comorbidities like; sepsis, RDS, birth asphyxia, meconium aspiration, intestinal obstruction, congenital infection, neonates received IVIG, conjugated (direct) hyperbilirubinemia suggestive of cholestasis or hepatobiliary disease (direct bilirubin >20% of total or >2 mg/dL); severe hemolytic disease requiring exchange transfusion; or documented exposure to probiotic supplementation prior to enrollment, and when legal guardians refused to participate or withdraw from the study.

A consecutive sampling method was used, enrolling all eligible neonates during the study period until the target sample size was achieved. Sample size was estimated using a two- sided comparison of mean rate of bilirubin decline between probiotic and non-probiotic groups. Assuming a clinically meaningful difference of 1.0 mg/dL/day in total serum bilirubin (TSB) decline, a standard deviation of 2.0 mg/dL/day (based on prior studies (13, 14)), $\alpha = 0.05$, and power = 80%, at least 64 infants per exposure group were required.

Allowing for approximately 20% attrition and subgroup analyses by PT status, a final target of 156 neonates was set and achieved.

2.5 Data Collection

Data were prospectively collected using a structured case report form. At enrollment, demographic and perinatal information (age in days, gestational age, birth weight, sex, mode of delivery) and feeding practices (exclusive breastfeeding, exclusive formula, mixed feeding) were recorded, along with established risk factors for neonatal jaundice (ABO incompatibility, Rh incompatibility, G6PD deficiency, β-thalassemia trait). Probiotic supplementation with a standardized Lactobacillus + Bifidobacterium preparation, specifically Lactobacillus acidophilus & Bifidobacterium lactis, given per uniform hospital protocol; (1 billion & 0.5 billion CFU, respectively daily for 10 days) defined the probiotic group, while infants not receiving probiotics served as controls. Decisions regarding PT were made independently by treating physicians according to institutional guidelines.

The clinical and laboratory evaluations were executed on Days 1, 2, 3, 5, and 7. Patients underwent venous TSB and PCV evaluations during each of these days to provide a means of assessing the kinetics of bilirubin and

hematologic status. The early (Days 1 –3) and late (Days 4–7) phases of bilirubin kinetics were prospectively defined and then the average daily rates of change in TSB (mg/dL/day) were determined by time period. The primary endpoint of this study was TSB decline rates during the early and late phases of bilirubin kinetics; moreover, the secondary endpoints of this study included peak bilirubin levels, PCV trends, rates of severe hyperbilirubinemia (TSB >15 mg/dL), polycythemia (PCV >65%), TSB <12 mg/dL, and clinical events or complications that required a change in level of care.

2.6 Ethical Considerations

Approval for the study was given by the Institutional Review Board at the University of Sulaimani prior to conducting the research, and all ethical guidelines of the Declaration of Helsinki, along with Good Clinical Practice, were adhered to during the conduct of the research. (No:369; Date: 3/11/2024). The researcher obtained written informed consent from all parents/legal guardians after explaining to them the purpose of the study, how the study would be carried out, and any potential risks. At this time, all families were guaranteed that they could withdraw from participation in the study at any time without affecting their clinical care. After 50% of participants had been recruited into the study, an independent Data Safety Monitoring Board (DSMB)evaluated safety data to continue monitoring participants' safety

2.7 Statistical Analysis:

The statistical analysis was completed by means of SPSS (version 27). Continuous variables were summarized with their means ± standard deviations, and comparisons between four groups were calculated using one-way ANOVA with an appropriate post hoc test. Categorical variables were reported as frequencies and percentages; comparisons between category variables were accomplished via chi-square or Fisher's exact test. Longitudinal PCV trends were assessed with repeated-measures ANOVA, and TSB changes over time (days 1–3 and 4–7) were analyzed as absolute decline and rate of change, using independent-samples t-tests stratified by PT and probiotic status. Multiple linear regression was used to identify independent predictors of TSB rate of change in early and late periods, including probiotic use, PT need, their interaction, birth weight, and initial TSB; model performance was evaluated with R^2 and adjusted R^2. Subgroup analyses compared TSB decline between probiotic and non-probiotic neonates by feeding type, risk status, and PT status, reporting mean differences with 95% confidence intervals. A two- sided p-value <0.05 was considered statistically significant

3. Results:

Table 1 presents the demographic and clinical characteristics of the four study groups. The mean age of neonates in days was comparable across groups (4.83 ± 2.06, 4.67 ± 1.86, 4.26 ± 1.45, and 4.95 ± 1.88 days, respectively) (p = 0.245), with no statistically significant difference. Similarly, there was no significant difference in mean gestational age, which was 36.58 ± 1.06 weeks, 36.87 ± 1.15 weeks, 36.71 ± 2.23 weeks, and 37.20 ± 1.82 weeks in the PT + Probiotic, PT + No Probiotic, No PT + No Probiotic, and No PT + Probiotic groups, respectively (p = 0.185) with no statistically significant difference. Regarding sex distribution, males constituted 17 (70.8%) in was statistically significant (p = 0.035). Mode of delivery did not differ significantly between groups.

Table 1. Demographic and Clinical Characteristics of the Study Groups.

Characteristic		PT + Probiotic (n=24)	PT + No Probiotic (n=24)	No PT + No Probiotic (n=38)	No PT + Probiotic (n=70)	p-value
Age (days)		4.83 ± 2.06	4.67 ± 1.86	4.26 ± 1.45	4.95 ± 1.88	0.245
Gestational age (weeks)		36.58 ± 1.06	36.87 ± 1.15	36.71 ± 2.23	37.20 ± 1.82	0.185
Birth weight (g)		2995 ± 298	3067 ± 244	3216 ± 317	3240 ± 495	0.025
Sex	Male	17 (70.8%)	18 (75.0%)	16 (42.1%)	44 (62.9%)	0.035
	Female	7 (29.2%)	6 (25.0%)	22 (57.9%)	26 (37.1%)	
Delivery mode	Vaginal	7 (29.2%)	12 (50.0%)	10 (26.3%)	24 (34.3%)	0.265
	Cesarean	17 (70.8%)	12 (50.0%)	28 (73.7%)	46 (65.7%)	

Table 2 summarizes feeding practices and risk factors among the four study groups. Feeding type differed significantly between groups (p = 0.012), breastfeeding was reported in 6 (25.0%), 12 (50.0%), 14 (36.8%), and 18 (25.7%) neonates, formula feeding in 0(0.0%), 3 (12.5%), 8 (21.1%), and 20 (28.6%) neonates, and mixed feeding in 18 (75.0%), 9 (37.5%), 16 (42.1%), and 32 (45.7%) neonates in the PT + Probiotic, PT + No Probiotic, No PT + No Probiotic, and No PT + Probiotic groups, respectively. In contrast, the distribution of risk factors did not differ significantly between groups (p = 0.125).

Table 2. Feeding Practices and Risk Factors.

Characteristic		PT + Probiotic	PT + No Probiotic	No PT + No Probiotic	No PT + Probiotic	p-value
Feeding type	Breastfeeding	6 (25.0%)	12 (50.0%)	14 (36.8%)	18 (25.7%)	0.012
	Formula	0 (0.0%)	3 (12.5%)	8 (21.1%)	20 (28.6%)	
	Mixed	18 (75.0%)	9 (37.5%)	16 (42.1%)	32 (45.7%)	
Risk factors	None	15 (62.5%)	15 (62.5%)	32 (84.2%)	44 (62.9%)	0.125
	ABO incompatibility	6 (25.0%)	5 (20.8%)	3 (7.9%)	18 (25.7%)	
	Rh incompatibility	3 (12.5%)	4 (16.7%)	3 (7.9%)	3 (4.3%)	
	β-thalassemia trait	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (4.3%)	
	G6PD deficiency	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.9%)	

Table 3 presents the initial laboratory parameters of the study groups. The mean initial TSB level was 14.88 ± 1.41 mg/dL in the PT + Probiotic group, 14.81 ± 1.25 mg/dL in the PT + No Probiotic group, 12.60 ± 0.70 mg/dL in the No PT + No Probiotic group, and 12.20 ± 0.75 mg/dL in the No PT + Probiotic group, with a highly significant difference among groups (p < 0.001). The mean initial packed cell volume (PCV) was 63.67 ± 7.63%, 59.58 ± 8.24%, 58.61 ± 3.94%, and 59.25 ± 6.50% in the PT + Probiotic, PT + No Probiotic, No PT + No Probiotic, and No PT + Probiotic groups, respectively, also showing a statistically significant difference between groups (p = 0.032).

Table 3. Initial Laboratory Parameters.

Parameter	PT + Probiotic	PT + No Probiotic	No PT + No Probiotic	No PT + Probiotic	p-value
Initial TSB (mg/dL)	14.88 ± 1.41	14.81 ± 1.25	12.60 ± 0.70	12.20 ± 0.75	<0.001
Initial PCV (%)	63.67 ± 7.63	59.58 ± 8.24	58.61 ± 3.94	59.25 ± 6.50	0.032

Table 4 summarizes the mean TSB decline over time according to PT and probiotic exposure. During days 1–3, the mean TSB decline was 4.25 ± 0.85 mg/dL in the PT + Probiotic group and 4.30 ± 0.82 mg/dL in the PT + No Probiotic group, versus 2.15 ± 0.65 mg/dL in the No PT + Probiotic group and 2.10 ± 0.68 mg/dL in the No PT + No Probiotic group; there was no significant difference between probiotic and no probiotic within either the PT group (p = 0.812) or the no-PT group (p = 0.721). In contrast, during days 4–7, the mean TSB decline was 1.20 ± 0.45 mg/dL in the PT + Probiotic group and 1.25 ± 0.42 mg/dL in the PT + No Probiotic group, compared with 2.85 ± 0.55 mg/dL in the No PT + Probiotic group and 1.40 ± 0.50 mg/dL in the No PT + No Probiotic group; although there was still no significant difference between probiotic and no probiotic among neonates receiving PT (p = 0.682), probiotic use was associated with a significantly greater decline among those not receiving PT (p < 0.001; statistically significant). Within-group comparisons showed that, in all four groups, the early decline (days 1–3) was significantly greater than the late decline (days 4–7) (all p < 0.001; statistically significant).

Table 4. Mean TSB Decline (mg/dL) According to PT and Probiotic Exposure.

Time Period	PT + Probiotic	PT + No Probiotic	No PT + Probiotic	No PT + No Probiotic	p-value*
Day 1–3 decline	4.25 ± 0.85	4.30 ± 0.82	2.15 ± 0.65	2.10 ± 0.68	PT: 0.812 No PT: 0.721
Day 4–7 decline	1.20 ± 0.45	1.25 ± 0.42	2.85 ± 0.55	1.40 ± 0.50	PT: 0.682 No PT: <0.001
Within-group significance†	p<0.001	p<0.001	p<0.001	p<0.001	—

*Comparison of probiotic vs. no probiotic within PT strata
†Early vs. late decline

Table 5 summarizes the multiple linear regression analysis of factors associated with the rate of change in TSB during days 1–3 and days 4–7. During days 1–3, probiotic use was not significantly associated with TSB rate of change (β = 0.085, 95% CI: –0.050 to 0.220; p = 0.215; not significant), nor was PT need (β = –0.296, 95% CI: –0.684 to 0.091; p = 0.133; not significant) or the probiotic × PT interaction term (β = –0.125, 95% CI: –0.385 to 0.135; p = 0.345; not significant). Birth weight showed no significant effect in this early period (β = 0.000, 95% CI: 0.000 to 0.000; p = 0.105; not significant), whereas higher initial TSB was significantly associated with a faster early decline (β = –0.354, 95% CI: – 0.454 to –0.254; p < 0.001; statistically significant). The model explained 54.1% of the variance in the day 1–3 TSB rate (R^2 = 0.541; adjusted R^2 = 0.526). In contrast, during days 4–7, probiotic use emerged as a strong positive predictor of TSB rate of change (β = 0.420, 95% CI: 0.280 to 0.560; p < 0.001; statistically significant), while PT need remained non-significant (β = –0.080, 95% CI: –0.220 to 0.060; p = 0.260; not significant). The probiotic × PT interaction term was significantly negative (β = –0.350, 95% CI: –0.520 to –0.180; p = 0.001; statistically significant), indicating that the effect of probiotics on late TSB decline differed according to PT status. Birth weight was also a significant predictor in this later period (β = 0.000, 95% CI: 0.000 to 0.000; p = 0.008;

statistically significant), whereas initial TSB was not (β = –0.025, 95% CI: –0.085 to 0.035; p = 0.415; not significant). The day 4–7 model accounted for 38.5% of the variance in TSB rate of change (R^2 = 0.385; adjusted R^2 = 0.362).

Table 5: Multiple Linear Regression Analysis of Factors Associated with TSB Rate of Change

Variable	Day 1–3 TSB Rate, Beta (95% CI)	p-value	Day 4–7 TSB Rate, Beta (95% CI)	p-value
Probiotic use	0.085 (–0.050 to 0.220)	0.215	0.420 (0.280 to 0.560)	<0.001
PT need	–0.296 (–0.684 to 0.091)	0.133	–0.080 (–0.220 to 0.060)	0.260
Probiotic × PT interaction	–0.125 (–0.385 to 0.135)	0.345	–0.350 (–0.520 to –0.180)	0.001
Birth weight	0.000 (0.000 to 0.000)	0.105	0.000 (0.000 to 0.000)	0.008
Initial TSB	–0.354 (–0.454 to –0.254)	<0.001	–0.025 (–0.085 to 0.035)	0.415
Model R^2 / Adjusted R^2	0.541 / 0.526	—	0.385 / 0.362	—

Table 6 describes the longitudinal trends in hematocrit (PCV) over the 7 -day follow-up across the four study groups (PT + Probiotic, PT + No Probiotic, No PT + Probiotic, and No PT + No Probiotic). In the PT + Probiotic group, mean PCV decreased from 63.67 ± 7.63% on day 1 to 52.13 ± 6.30% on day 7 (p-trend < 0.001). Similarly, in the PT + No Probiotic group, PCV declined from 59.58 ± 8.24% on day 1 to 49.29 ± 5.26% on day 7 (p-trend < 0.001). In the No PT + Probiotic group, PCV fell from 59.25 ± 6.50% on day 1 to 51.30 ± 4.75% on day 7 (p-trend < 0.001), while in the No PT + No Probiotic group, it decreased from 58.61 ± 3.94% on day 1 to 52.71 ± 5.92% on day 7 (p-trend < 0.001).

Table 6. Hematocrit (PCV) Trends Over Time by Study Group.

Time Point	PT + Probiotic	PT + No Probiotic	No PT + Probiotic	No PT + No Probiotic
Day 1	63.67 ± 7.63	59.58 ± 8.24	59.25 ± 6.50	58.61 ± 3.94
Day 2	59.75 ± 5.72	56.50 ± 7.08	57.10 ± 5.85	57.13 ± 4.75
Day 3	58.04 ± 3.90	56.75 ± 3.88	55.80 ± 4.05	54.90 ± 4.46
Day 4	53.78 ± 2.74	52.75 ± 4.28	52.45 ± 3.40	52.91 ± 3.26
Day 7	52.13 ± 6.30	49.29 ± 5.26	51.30 ± 4.75	52.71 ± 5.92
p-trend	<0.001	<0.001	<0.001	<0.001

Table 7 presents the subgroup analysis of the effect of probiotics on TSB kinetics, expressed as rate differences between probiotic and non-probiotic groups during days 1 – 3 and days 4–7. Overall (n = 156), probiotics did not significantly affect the early TSB rate (day 1–3 rate difference = 0.06, 95% CI: –0.08 to 0.20; p = 0.385; not significant), whereas they were associated with a significantly greater late TSB decline (day 4 –7 rate difference = 0.32, 95% CI: 0.18 to 0.46; p < 0.001; statistically significant). This pattern was consistent across feeding subgroups, with no significant early effect in exclusively breastfed neonates (n = 47; 0.10, 95% CI: –0.12 to 0.32; p = 0.365), formula-fed neonates (n = 30; 0.22, 95% CI: –0.02 to 0.46; p = 0.072), or mixed-fed neonates (n = 79; 0.01, 95% CI: –0.15 to 0.17; p = 0.890; not significant), but a significantly greater day 4 –7 decline in all three groups: 0.28 (95% CI: 0.08 to 0.48; p = 0.008) in breastfed, 0.45 (95% CI: 0.22 to 0.68; p < 0.001) in formula-fed, and 0.25 (95% CI: 0.08 to 0.42; p = 0.005) in mixed- fed infants (all statistically significant). A similar pattern was observed when stratified by risk status: in high-risk neonates (n = 52), the early rate difference was not significant (0.18, 95% CI: –0.05 to 0.41; p = 0.118), but the late rate difference was significant (0.35, 95% CI: 0.15 to 0.55; p = 0.001); in low-risk

neonates (n = 104), the early effect remained non-significant (0.02, 95% CI: –0.13 to 0.17; p = 0.790), whereas the day 4–7 rate difference was significant (0.28, 95% CI: 0.12 to 0.44; p = 0.001). In the PT subgroup (n = 48), probiotics had no significant impact on either early (–0.02, 95% CI: –0.22 to 0.18; p = 0.850; not significant) or late TSB rate (0.05, 95% CI: –0.10 to 0.20; p = 0.520). By contrast, among neonates who did not receive PT (n = 108), probiotic use did not alter the early rate (0.08, 95% CI: –0.10 to 0.26; p = 0.385) but was associated with a significantly greater TSB decline during days 4–7 (0.38, 95% CI: 0.25 to 0.51; p < 0.001).

Table 7: Effect of Probiotics on TSB Kinetics in Clinical Subgroups.

Subgroup	n	Day 1–3 Rate Difference	95% CI	p-value	Day 4–7 Rate Difference	95% CI	p-value
Overall	156	0.06	(–0.08 to 0.20)	0.385	0.32	(0.18 to 0.46)	<0.001
Breastfeeding	47	0.10	(–0.12 to 0.32)	0.365	0.28	(0.08 to 0.48)	0.008
Formula feeding	30	0.22	(–0.02 to 0.46)	0.072	0.45	(0.22 to 0.68)	<0.001
Mixed feeding	79	0.01	(–0.15 to 0.17)	0.890	0.25	(0.08 to 0.42)	0.005
High risk	52	0.18	(–0.05 to 0.41)	0.118	0.35	(0.15 to 0.55)	0.001
Low risk	104	0.02	(–0.13 to 0.17)	0.790	0.28	(0.12 to 0.44)	0.001
PT	48	–0.02	(–0.22 to 0.18)	0.850	0.05	(–0.10 to 0.20)	0.520
No PT	108	0.08	(–0.10 to 0.26)	0.385	0.38	(0.25 to 0.51)	<0.001

Table 8 summarizes peak bilirubin levels and key clinical outcomes across the four study groups. Peak TSB was significantly higher in the PT groups compared with the non-PT groups, with mean values of 14.85 ± 1.40 mg/dL in the PT + Probiotic group, 14.82 ± 1.38 mg/dL in the PT + No Probiotic group, 11.85 ± 0.75 mg/dL in the No PT + Probiotic group, and 12.60 ± 0.70 mg/dL in the No PT + No Probiotic group (p < 0.001). The mean time to achieve TSB <12 mg/dL also differed significantly, being 3.2 ± 0.8 days, 3.3 ± 0.9 days, 2.8 ± 0.6 days, and 4.1 ± 0.9 days in the PT + Probiotic, PT + No Probiotic, No PT + Probiotic, and No PT + No Probiotic groups, respectively (p < 0.001), indicating faster resolution in the No PT + Probiotic group and slower resolution in the No PT + No Probiotic group. Peak PCV was significantly different between groups (p = 0.008), with mean values of 64.2 ± 7.1%, 61.1 ± 5.4%, 59.8 ± 5.2%, and 59.1 ± 4.1% in the PT + Probiotic, PT + No Probiotic, No PT + Probiotic, and No PT + No Probiotic groups, respectively. Polycythemia (PCV >65%) occurred in 9 (37.5%) neonates in the PT + Probiotic group, 4 (16.7%) in the PT + No Probiotic group, 5 (7.1%) in the No PT + Probiotic group, and 3 (7.9%) in the No PT + No Probiotic group, with a statistically significant difference across groups (p = 0.018). Severe hyperbilirubinemia (TSB >15 mg/dL) was observed in 11 (45.8%) and 10 (41.7%) neonates in the PT + Probiotic and PT + No Probiotic groups, respectively, and in 0 (0.0%) neonates in both No PT + Probiotic and No PT + No Probiotic groups, representing a highly significant difference between PT and non-PT strata (p < 0.001).

Table 8. Peak Bilirubin Levels and Clinical Outcomes.

Outcome	PT + Probiotic (n=24)	PT + No Probiotic (n=24)	No PT + Probiotic (n=70)	No PT + No Probiotic (n=38)	p-value
Peak TSB (mg/dL)	14.85 ± 1.40	14.82 ± 1.38	11.85 ± 0.75	12.60 ± 0.70	<0.001
Time to TSB <12 mg/dL (days)	3.2 ± 0.8	3.3 ± 0.9	2.8 ± 0.6	4.1 ± 0.9	<0.001
Peak PCV (%)	64.2 ± 7.1	61.1 ± 5.4	59.8 ± 5.2	59.1 ± 4.1	0.008
Polycythemia (PCV >65%)	9 (37.5%)	4 (16.7%)	5 (7.1%)	3 (7.9%)	0.018
Severe hyperbilirubinemia (TSB >15 mg/dL)	11 (45.8%)	10 (41.7%)	0 (0.0%)	0 (0.0%)	<0.001

4. Discussion

The primary aim of the current research was to investigate whether giving probiotics would have an impact on the UCB levels found in neonates who had been diagnosed with hyperbilirubinemia (UHB). This was accomplished through a prospective clinical trial performed on 156 UHB neonates and showed significant reductions in bilirubin levels and at a quicker pace (between days 1-3) (between days 4-7), from when probiotics were given to the group of neonates receiving PT. A statistical analysis was conducted using multivariate regression, which demonstrated that while the probiotics had a significant effect on the accelerated reduction of bilirubin after day four, there was no difference in bilirubin level reductions between the two treatment groups (i.e., probiotics versus non- probiotics) when compared with PT. The current study also showed that when probiotics were given to non-PT neonates, the time required to achieve bilirubin levels below 12 mg/dL was significantly shorter. However, the incidence of severe hyperbilirubinemia was restricted to PT neonates only.

Findings showed variations of feeding patterns across the sample populations within the research design. A high proportion of the sample population (75.0%) classified as receiving mixed feed were in the group that received probiotics, PT plus Probiotics. Breast milk provides oligosaccharides which serve as prebiotics, thus aiding in the proliferation of good bacteria (17). The presence of these 'good' bacteria diminishes the activity of beta-glucuronidase, which enhances the elimination of bilirubin through the reduction of enterohepatic circulation (18).

A main finding of this study was that probiotic supplementation provided during therapy's first three days had no significant effects on bilirubin reduction regardless of whether the patient had or did not have PT or was receiving PT. Fellow researchers have previously reported similar conclusions from their own studies regarding the timing of maximal efficacy of PT during the first several days. Thus, the above sample provides evidence that probiotics have insufficient time to produce a biological effect on bilirubin during the first three days of treatment and would be expected to not produce an effect on bilirubin (19-21). From a physiologic standpoint, probiotics take time to colonize the gut and therefore should not be expected to produce an instantaneous change in the enterohepatic circulation of bilirubin (12, 22). However, during the second week of treatment (days 4-7), there was evidence that the use of probiotics was associated with a clinically important decrease in bilirubin in the non-PT group of neonates. This finding is one of the most important outcome measures of this study, and corroborates earlier studies regarding use of probiotics and decreased enterohepatic recirculation of bilirubin (16, 23).

These findings are in agreement with some previous studies on the use of probiotics for the

management of neonatal hyperbilirubinemia, yet notable differences are also evident. In a double-blind RCT by Tsai et al. involving 300 neonates in Taiwan, the combination of PT and two types of probiotics reduced the duration of PT and accelerated the rate of bilirubin decline. In one group, the length of hospitalization was also shortened by 7 hours, and the safety profile was similar to that observed in the present study (no serious adverse events). However, differences in outcomes may be attributable to the specific probiotic strains used, which may differentially affect the enterohepatic cycle of bilirubin (24). In the present study, the lack of difference in TSUB may be due to the immaturity of the gut flora or interactions with PT.

The study by Ahmadipour et al. on 83 neonates in Iran examined the effect of synbiotics (a combination of probiotics and prebiotics) and reported a significant reduction in bilirubin levels, increased frequency of urination and defecation, and a shorter duration of hospitalization in the synbiotic group. The aforementioned data support other positive studies; however, in the current study, no discernible differences in TSUB or PCV were observed. One possible explanation for these results is the treatment with synbiotics instead of single-culture probiotics may provide a synergistic effect on the intestinal microbiota. In addition, the referenced study included exclusively breastfed neonates, while the current study included some individuals breastfed and other individuals receiving mixed feedings which can alter the level of absorption of probiotics. Differences in study design (RCT vs. cohort), could also introduce selection bias to the current study (25).

A comprehensive examination of data through multiple regression analysis determined the existence of a strong significant negative interaction between supplemental probiotics and preventive treatment (PT) on days 4 through 7. The negative coefficient associated with probiotic supplementation when PT was provided demonstrates that the effect of probiotics on bilirubin levels observed in participants who received no PT would be markedly reduced or eliminated if those same participants were treated with PT. The reason for this reduced or eliminated effect was due to the ceiling effect, wherein a highly efficacious medical intervention (such as PT) reduces the amount of bilirubin present in the body to the extent that it no longer exists in the gastrointestinal tract and converts it to the excreted form in the skin (26, 27).

Bilirubin concentrations observed in probiotic subjects were lower on days 4 and 7 of the study by Ramadan Kamel et al. out of Egypt when compared to their initial value; this parallel finding lends support to a significant decrease in TSUB over time as documented within this present study. Additionally, both studies showed that there were no medically serious adverse effects related to the consumption of probiotics: an indication of the safety of probiotics overall (28). A previously completed clinical trial by Ain et al. out of Pakistan has also shown that there were significantly higher reductions of bilirubin among probiotic participants as opposed to those receiving placebo, and additionally no significant difference was observed between the two groups in terms of reported adverse effects (29).

The research results indicate that neonates who did not receive PT experienced a noteworthy speed of recovery as evidenced by the significantly less time taken for the mean TSB to fall below 12mg/dL with probiotics (i.e., 2.8 ± 0.6 days) than without probiotics (i.e., 4.1 ± 1.9 days). The difference between the groups was 1.3 days, an important gain that would facilitate prompt and effective treatment of jaundice and lessen the economic impact on the health care system. Probiotics could therefore be considered a valuable and practical option for treating physiological jaundice on an outpatient basis within the community where the participants resided.

The probiotic's ability to reduce TSB decline

from day four to days seven was consistent across all studied groups. Late phase TSB decline was statistically significantly improved for the probiotic group compared to the control in those neonates exclusively breastfed, fed only formula, or received a combination of both types of feeding; this makes the lack of statistical differences on TSB decline between feeding types surprising as it indicates that the probiotic strain studied must be capable of colonizing and functioning in different neonatal gut environments.

Probiotics have also demonstrated an effect on the prolongation of action in neonates because of their mechanism, which reduces enterohepatic cycling. This reduction may be useful in treating hematological risk factors, even though probiotics themselves do not directly affect physiological processes associated with hematology (30).

The findings related to the packed cell volume (PCV) presented a thorough understanding of the hematology processes of the neonate and appeared to be unaffected by probiotic therapy. The total PCV declined significantly over the 7 days of follow-up in all four study groups. This indicates that probiotics do not influence hemolytic processes, which are one of the predominant sources of bilirubin production (31). These results are also consistent with the mechanisms by which probiotics have been proposed to have an impact, which are primarily related to the gastrointestinal tract and the enterohepatic circulation of bilirubin.

This study has a number of strengths that build on the current body of knowledge. The prospective design allows researchers to collect high quality, accurate data. The large sample size is also a significant strength that adds to the study's statistical power compared with previously conducted studies within this field. There are some limitations to this study, including a lack of randomization, insufficient information about the specifics of a probiotic intervention, no long-term follow up for adverse events, and not fully controlling for PT rates.

3. Conclusion

This study establishes the usefulness of using probiotics with other treatments for neonatal UHB in a cohort of neonates. The main findings of this research support that the action of probiotics is dependent on when they are given. Probiotics will only be effective if given after colonization of the intestines, generally indicated to be after four days of age. It was found that in newborns with mild or moderate jaundice who do not receive phototherapy, probiotics will positively affect the rate of TSB decrease during the later portion of phototherapy; they will also decrease the length of time until bilirubin levels are within normal limits, i.e., requiring less than one day less than with phototherapy alone. This supports probiotics being able to significantly disrupt the enterohepatic circulation of bilirubin. However, in extreme cases resulting in requiring phototherapy as the main treatment modality, the effect of probiotics may be statistically minimal, thus confirming the hypothesis of a "ceiling effect" of PT.

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