ORIGINAL ARTICLE

Comparison of the effect of ursodeoxycholic acid and multistrain synbiotic on indirect hyperbilirubinemia among neonates treated with phototherapy: A double-blind, randomized, placebo-controlled clinical trial study

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Background: This study was aimed at evaluating the effect of ursodeoxycholic acid (UDCA) and multistrain synbiotic on indirect hyperbilirubinemia among neonates treated with phototherapy. **Materials and Methods:** This double-blind, randomized clinical trial was conducted on 120 subjects presenting with indirect hyperbilirubinemia in 2019. Subjects were randomly divided into three groups of synbiotic, UDCA, and control. The synbiotic group received five drops/day of synbiotic in addition to phototherapy. UDCA group received 10 mg/kg/day of Ursobil divided every 12 h in addition to phototherapy. The Control group received a placebo (water) in addition to phototherapy. Phototherapy was discontinued when the bilirubin levels reached <10 mg/dL. Total bilirubin levels were measured using the diazo method at 12, 24, and 36 h after hospitalization. This study used repeated measure analysis of variance and post hoc tests. **Results:** The mean total of bilirubin was substantially decreased in both synbiotic and UDCA groups as compared to the control group at 24 h after hospitalization ($P < 0.001$). Moreover, the Bonferroni post hoc test showed significant differences regarding the mean total of bilirubin between the three groups ($P < 0.05$) except for the association between UDCA and synbiotic at 24 h after hospitalization ($P > 0.99$). **Conclusion:** Findings suggest that UDCA and synbiotic administration alongside phototherapy are more effective in reducing bilirubin levels as compared to phototherapy alone.

Key words: Hyperbilirubinemia, jaundice, neonatal, neonatal, symbiotic, ursodeoxycholic acid

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INTRODUCTION

Neonatal jaundice is characterized by the yellow color of the skin and eyes of infants. It is an inescapable and common phenomenon during the 1st week of life which occurs in 60% and 80% of term and preterm newborns, respectively.[1-3] The most common treatment for reducing bilirubin levels is phototherapy in which with blue or severe white light exposure to the baby, the indirect bilirubin is converted to water-soluble compounds and excreted in the bile and then is repelled by the liver from the baby's body.[4,5] Phototherapy has side effects such as dehydration, skin rashes, watery diarrhea, transient tanning effect, and blue baby syndrome. Moreover, many hypotheses have been raised about the increased risk of melanoma progression during phototherapy.[6-8]

Ursodeoxycholic acid (UDCA) is a bile acid widely used in the treatment of cholestatic liver disease. It protects

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the liver against oxidative stress, prevents cell apoptosis, stimulates blood flow to bile, and suppresses destructive factors in immunological mechanisms.[9] Multiple mechanisms are thought to be involved in the entry of bilirubin into the brain. These include the following: (i) excessive production of bilirubin surpasses the blood and tissues' normal buffering capacity; (ii) altered bilirubin binding potential, resulting in unconjugated bilirubin in circulation; (iii) permeability of the central nervous system due to disruption of the blood–brain barrier and (iv) other factors that affect or act independently.[10] About 90% of the drug is absorbed through the small intestine. It has a high degree of protein binding and is metabolized in the liver; the drug is excreted mostly through the stool and to a small extent through the urine. It is well tolerated and there are few reported side effects among infants.[9,11]

For the first time, the synbiotic term was used to describe a mixture of probiotics and prebiotics about 20 years ago. Synbiotics are usually a combination of *Bifidobacterium* and fructooligosaccharides (FOS) or *Lactobacillus rhamnosus* and inulin or *Bifidobacterium* and *lactobacillus* with FOS or inulin.[12] Given that probiotics are useful as intestinal bacteria and prebiotics as sugar substrates have a beneficial role for these bacteria by reducing the duration of material transfer in the intestine and accelerating the excretion of bilirubin in the feces and reducing the enterohepatic cycle. Furthermore, they are effective in the treatment of infantile colic and in the prevention of sepsis (exclusively on preterm infants) and have no adverse effect on infant health.[4,13,14]

Given the high prevalence of neonatal jaundice and the emotional pressures inflicted on the mother and neonate during phototherapy separation and to reduce the cost and length of hospitalization, adjuvant therapy alongside phototherapy is needed to accelerate the treatment of neonatal jaundice. To the best of our knowledge, no study has been conducted to evaluate the effect of UDCA and synbiotic together on indirect hyperbilirubinemia among neonates; thus, the aim of this study was to assess the therapeutic effect of UDCA and multistrain synbiotic administration simultaneously on indirect hyperbilirubinemia among neonates treated with phototherapy.

SUBJECTS AND METHODS

This double-blind, randomized, placebo-controlled clinical trial study with parallel groups was conducted to assess the effect of UDCA and multistrain synbiotic administration on indirect hyperbilirubinemia among neonates treated with phototherapy in 2019.

Subjects

The study population under study included all the neonates presenting with indirect hyperbilirubinemia referred to the neonatal clinic of Heshmatiyeh Hospital, Sabzevar, Iran from 30 January 2019 to 30 March 2019. Subjects who met the inclusion criteria such as neonates with 2–14 days of age without hemolytic icterus, weight over 2500 g, term (gestational age >37 weeks), healthy, exclusive breastfeeding, normal range of reticulocyte count, indirect bilirubin between 14 and 25 mg/dL and direct bilirubin of <2 mg/dL and with no hematoma cerebral and caput succedaneum, without polycythemia (hematocrit over 65), comorbidity, and exchange transfusion were included. Subjects with low hemoglobin (Hb) levels, glucose-6-phosphate dehydrogenase (G6PD or G6PDH), positive Coombs test, receiving serum therapy, antibiotic therapy, and intravenous immunoglobulin as well as a neonate with abnormal peripheral blood smear tests, septicemia, Crigler–Najjar syndrome, Gilbert syndrome, thyroid, and liver diseases, with direct hyperbilirubinemia, and ABO blood and Rh incompatibility as well as being neonate of a diabetic mothers were excluded.

Clinical assessment

Eligible neonates were randomly divided into three groups of multistrain synbiotic, UDCA, and control. The synbiotic group was administered a maximum daily dose of five drops of synbiotic in addition to phototherapy (PediLact® drop; provided by Zist Takhmir Co., Tehran, Iran). PediLact® is a synbiotic compound consisting of high levels of the three beneficial bacterial strains namely *L. rhamnosus* (CBT LR5 2 × 10^9 CFU), *Lactobacillus reuteri* (CBT LR3 2 × 10^9 CFU), and *Bifidobacterium infantis* (CBT BI3 2 × 10^9 CFU) as well as FOS as a prebiotic component. UDCA group was administered a dose of 10 mg. kg^{-1}. Day^{-1} of Ursobil divided two times per day in addition to phototherapy (UDCA, capsule 300 mg; provided by Alborz Darou Co., Tehran, Iran). The UDCA was diluted with water and sucked by neonates. The control group received a placebo (water) in addition to phototherapy. Phototherapy was performed using a device from NeoMedLight® company at 30–35 cm of neonates. It should be noted that phototherapy was stopped for allowing breastfeeding. The device consists of eight daylight fluorescent lamps (Tucson lamps; TL 20W/52 bulbs) with wavelengths of 425–475 nm. Neonatal eyes and genitals were covered before phototherapy. To increase the level of contact, neonates were regularly switched during phototherapy.

Outcomes

The main endpoint of this study was the measurement of total bilirubin levels using the orthodox diazo method at 12, 24, and 36 h after hospitalization. The phototherapy was discontinued whenever the total bilirubin level reached <10 mg/dL. Primary blood tests, including Hb, Hct, bilirubin total, direct reticulocyte, Phosphate-buffered saline (PBS), Coombs, BG, Rh, and G6PD were performed to determine the cause

of their jaundice. Furthermore, complete medical history and physical examination were recorded on the 1st day of hospitalization. The second endpoint of this study was the assessment of pain using the pain assessment tool (PAT). The PAT is a validated PAT that is used in the neonatal intensive care unit to assess pain levels in infants. In total, there are six parameters, four of which are behavioral and five of which are physiological. In the 10th parameter, nurses evaluate the pain of the infant according to their clinical judgment. A total of 0–20 is calculated by adding the scores, with higher scores indicating greater pain levels. An infant's face and whole body are observed by the clinician for 2 min without interruption while the assessment is being performed. Once the clinician observes the infant for 2 min, he or she uses a gentle touch to determine the tone or tension of the infant's muscles. As soon as the observation period is over, the parameters are scored.[15]

Sample size determination

According to the Honar *et al*.'s study,[16] the sample size was estimated to be 40 individuals in each group considering the effect size of 0.24, power of 80%, and a significance level of 0.05 as well as 10% as dropout rate.

Blinding and randomization

Eligible neonates were randomly assigned to three groups of multistrain synbiotic, UDCA, and control using a web-based random allocation system provided by the statistical consultant in block sizes of six. The nurse investigator (who blindly treated the subjects with drugs and observed the outcome) and parents were blissfully unaware of the study protocol and group assignment. We used 120 sequentially numbered, opaque, sealed envelopes that contained the neonates' group assignment. After being shuffled, all the envelopes were distributed among the parents. The physician assigned the neonates to the right treatment after opening the envelopes.

Ethical consideration

This study protocol was approved by the regional ethics committee of Sabzevar University of Medical Sciences with the code of IR.MEDSAB.REC.1397.094 and was registered at the Iranian Registry of Clinical Trials (ID: IRCT20181006041252N9). Parents or legal representatives of all neonates signed a written informed consent before inclusion and subjects' anonymity was preserved. No name and personnel identification were defined in the questionnaire.

Statistical analysis

All the continuous and categorical variables were presented as mean (± standard deviation) and frequency (percent), respectively. After checking the normality of data using Shapiro–Wilk test, the analysis of variance (ANOVA) test and Chi-square test, as appropriate, were used to compare the baseline demographic data. Moreover, the repeated measure ANOVA test was used to compare the mean total of bilirubin among the three groups, and the Bonferroni *post hoc* test was applied to adjust for multiple significance testing in the ANOVA test. Data analysis was performed using STATA (version 12, Stata Corp, College Station, Texas, USA) and sample size calculation was conducted using G*Power version 3.0.10. Statistical significance was set at $P < 0.05$.

RESULTS

In total, 120 eligible neonates were randomized, out of which, 120 subjects completed the study until the 12 h after hospitalization and 83 subjects until the 24 h after hospitalization after starting intervention [Figure 1]. The mean age of the subjects was 125.63 ± 54.88 years and 53.33 ($n = 64$) were male. All the demographic characteristics of the subjects are presented in Table 1.

As shown in Table 2, based on the ANOVA test, the mean total of bilirubin was substantially decreased in both synbiotic and UDCA groups as compared to the control group at 24 h after hospitalization ($P < 0.001$). Moreover, the Bonferroni *post hoc* test showed significant differences regarding the mean total of bilirubin between the three groups ($P < 0.05$) except for the association between UDCA and synbiotic at 24 h after hospitalization ($P > 0.99$). It should be mentioned that the reduction in bilirubin levels was greater in the UDCA group than in other groups at 12 h after hospitalization. Moreover, more than 75% of the subjects were reached <10 mg/dL and discharged at 36 h after hospitalization and only two subjects in the synbiotic group and 27 subjects in the control group remained.

Regarding tolerance of the treatment, we found no side effects, namely, diarrhea and vomiting in the UDCA group and we observed only mild abdominal pain in five subjects in the synbiotic group.

DISCUSSION

This study was aimed at comparing the effect of UDCA and multistrain synbiotic on indirect hyperbilirubinemia among neonates treated with phototherapy. The findings of this study showed that total bilirubin levels were significantly decreased after 24 h after hospitalization in both UDCA and synbiotic groups as compared with the control one. Moreover, a much more reduction in bilirubin levels was observed in the UDCA group as compared with other groups at 12 h after hospitalization.

A possible explanation of our results regarding the effectiveness of UDCA may derive from the three main

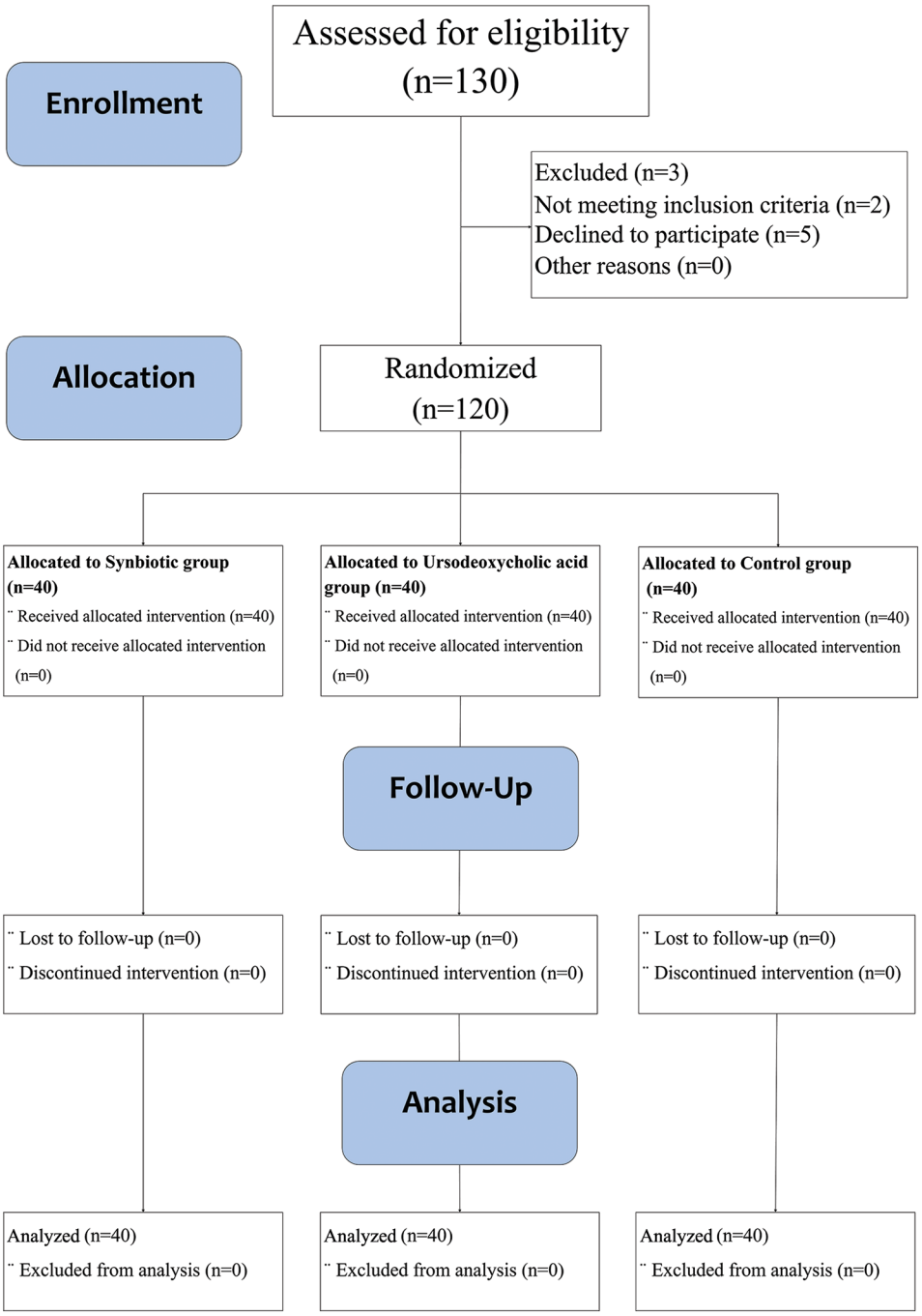

Figure 1: CONSORT diagram of the study

mechanisms of action for this drug, namely, (i) protection of cholangiocytes against the toxic effect of hydrophobic bile acids by reducing the toxicity of bile acids and decreasing their concentration against cholangiocytes, (ii) stimulation of bile flow secretion mainly by calcium and protein kinase C-alpha-dependent mechanisms and regulation of extracellular kinase signals that induce molecular carriers to the canalicular membrane of hepatocytes and possibly activate these mentioned carriers, and (iii) protection of hepatocytes against bile acid-induced apoptosis by transiently blocking mitochondrial membrane permeability and potentially stimulating survival pathways.[17] According to the results of our study, Ursobil resulted in a 24-h decrease in phototherapy duration in neonates with indirect hyperbilirubinemia most likely by increasing indirect bilirubin turnover through fecal excretion. In line with our results, Honar *et al*.'s[16] study showed a significant reduction in bilirubin levels after 24 h of hospitalization in neonates treated with phototherapy. Furthermore, a study by Shahramian *et al*.[18] (2019) found UDCA to be

Table 1: Baseline data characteristics of subjects by study group, mean±standard deviation, or *n* (%)

Parameter	Groups			*P***
	Synbiotic (*n*=40)	UDCA (*n*=40)	Control (*n*=40)	
Age (days)	119.40±45.99	124.45±61.23	133.05±56.80	0.535
Weight (g)	3102.75±312.99	3068.50±346.56	3103.00±344.10	0.869
Basal bilirubin (mg/dL)	17.34±2.15	16.67±2.08	17.28±2.23	0.311
ALOS† (h)	21.00±6.51	16.50±5.88	32.10±5.69	0.201
Gender (male)	27 (42.19)	20 (31.25)	17 (26.56)	0.071
Delivery type (vaginal)	26 (31.33)	28 (31.73)	29 (34.94)	0.761
Mother's Rh (positive)	39 (34.82)	36 (32.14)	37 (33.04)	0.392
Neonates' Rh (positive)	34 (33.01)	36 (34.95)	33 (32.04)	0.619
Mother's blood group				
A	16 (33.33)	14 (29.17)	18 (37.50)	0.393
B	2 (31.03)	12 (41.38)	8 (27.59)	
AB	0	4 (57.14)	3 (42.86)	
O	15 (41.67)	4 (27.78)	3 (30.56)	
Neonates' blood group				
A	11 (25.58)	12 (27.91)	20 (46.51)	0.158
B	9 (39.13)	10 (43.48)	4 (17.39)	
AB	3 (33.33)	5 (55.56)	1 (11.11)	
O	17 (37.78)	13 (28.89)	15 (33.33)	

**ANOVA test or Chi-square test as appropriate; †The average length of stay in hospitals. UDCA=Ursodeoxycholic acid; ALOS=Average length of stay

Table 2: Comparisons of the mean of total bilirubin among three groups

Time*	Subgroup (*n*) Mean±SD	Group (*n*) Mean±SD	Greenhouse-Geisser‡			Bonferroni *post hoc* test		
			Group (between effects)	Time (within effects)	Group×time	Synbiotic	UDCA	Control
12 h after hospitalization	Control (40) 13.62±1.89	Synbiotic (40) 11.61±2.62	<0.001	<0.001	0.029	-	0.001	0.001
		UDCA (40)† 9.53±2.65	<0.001			0.001	-	<0.001
						0.001	<0.001	-
24 h after hospitalization	Control (40) 10.64±2.35	Synbiotic (28) 7.68±2.12	<0.001	<0.001	<0.001	-	>0.99	<0.001
		UDCA (15) 7.00±2.19	<0.001			>0.99	-	<0.001
						<0.001	<0.001	-
36 h after hospitalization	Control (27) 8.96±1.20	Synbiotic (2) 7.10±0.42	0.041	<0.001	<0.001	-	-	0.041
		UDCA (0) 0.00±0.00	-			-	-	-
						0.041	-	-

*Total bilirubin was checked and recorded until 10 mg/dL; †UDCA; ‡Repeated measure ANOVA test. UDCA=Ursodeoxycholic acid; SD=Standard deviation; ANOVA=Analysis of variance

effective in decreasing total bilirubin levels in neonates with unconjugated hyperbilirubinemia under phototherapy, but this treatment was not effective in reducing direct bilirubin levels.

Previous studies have shown a much more reduction in bilirubin levels using synbiotic or pre/probiotic products. In this regard, Ahmadipour *et al*.'s[19] study investigated the effect of synbiotic on the treatment of jaundice in full-term neonates; the results showed a substantial reduction in indirect hyperbilirubinemia and length of hospital stay using synbiotic as adjuvant therapy. Moreover, Chen *et al*.'s[20] meta-analysis study revealed the effectiveness of probiotic supplementation among neonates presenting with pathologic jaundice. Furthermore, in a systematic review conducted by Deshmukh *et al*.,[21] the safety and efficacy of probiotic supplements on neonatal hyperbilirubinemia were evaluated and the results showed a significant reduction in phototherapy duration using probiotic supplementation in neonatal jaundice. The results of these studies agree with our results. Nonetheless, Armanian *et al*.'s[22] systematic review showed unreliable evidence regarding the efficacy of prebiotics on hyperbilirubinemia. A possible rationale for our results may stem from two main factors; (i) using multistrain synbiotic produced used in our study and (ii) running a well-designed study by minimizing the potential confounding factors. Considering the limitation of this study, further studies are needed to assess various stains of synbiotic products and implement long-term follow-up to confirm the tolerability and safety of the drugs among neonates.

Limitations and strengths

The main limitations of this study were as follows: (i) collecting data from single center which may influence our

results (ii) assessing the side effect resulting from the drugs in the short term; (iii) using available synbiotic product in our country which may affect the generalizability of our results as various synbiotic products existing globally. On the other hand, being the world's first clinical trial study to investigate the effect of UDCA and multistrain synbiotic simultaneously on indirect hyperbilirubinemia among neonates treated with phototherapy would be the strengths of this study.

CONCLUSIONS

Findings suggest that UDCA and synbiotic administration alongside phototherapy are more effective in reducing bilirubin levels as compared to phototherapy alone; however, the effect of UDCA administration on the photography duration and length of hospital stay is noticeably greater as compared to other groups at the 24 h after hospitalization.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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