Original Article

An Evaluation of Ursodeoxycholic Acid Treatment in Prolonged Unconjugated Hyperbilirubinemia due to Breast Milk

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Abstract

Background/Aim: Prolonged jaundice is one of the most common problems during neonatal period. The aim of this study was to evaluate the efficiency of ursodeoxycholic acid (UDCA) treatment in newborn infants with prolonged unconjugated hyperbilirubinemia. **Materials and Methods:** The present study included 27 patients who were fed by breast milk and followed up in the outpatient clinic due to prolonged jaundice without any underlying etiological factor; 10 mg/kg/day UDCA was administrated in two doses for 7 days. Furthermore, 20 newborns diagnosed with prolonged jaundice with same characteristics were enrolled as the control group. The control group was also given a placebo; demographic characteristics, liver functions tests before and after the treatment, bilirubin decrease rates, and hemogram parameters of groups were compared. **Results:** Total bilirubin levels in the study and control groups before the treatment were 16.02 ± 1.41 mg/dL and 15.93 ± 1.66 mg/dL, respectively (P = 0.84). Total bilirubin levels in the study and control groups at day 7 after UDCA treatment were detected 8.18 ± 2.31 mg/dL and 13.92 ± 2.66 mg/dL, respectively (P < 0.001), and at day 14 after the treatment were 5.45 ± 2.59 mg/dL and 11.91 ± 2.83 mg/dL, respectively (P < 0.001). Furthermore, serum aspartate aminotransferase (AST) was detected <21 U/L in the ROC analysis after UDCA treatment (P = 0.04). **Conclusion:** The study outcomes indicated that an efficient reduction in total bilirubin levels may be achieved, and outpatient clinic follow-up period may be reduced in patients whom UDCA was administrated. Moreover, it may be speculated that AST can be used to evaluate the efficacy after treatment. However, studies with larger sample sizes are needed for the routine use of UDCA in the treatment of prolonged jaundice.

Keywords: *Newborn, prolonged jaundice, ursodeoxycholic acid*

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Introduction

Bilirubin is a potential toxic catabolic product of hem metabolism; the main stages of the metabolism include bilirubin synthesis, transportation in the plasma, hepatic uptake, hepatic conjugation, excretion into the bile, and intestinal reabsorption. Any pathology at any stage causes an increase in the blood bilirubin level and jaundice appears.[1] Persistence of jaundice after 21 days in preterm newborns and after 14 days in term newborns (bilirubin >10 mg/dl) is defined as prolonged jaundice.[2] Prolonged jaundice is detected in 15% of all newborns and in up to 40% of those fed with breast milk.[3,4] This may indicate an underlying pathology. However, there is not any underlying cause in majority of the cases.[5]

Different drugs have been used for treatment of newborn jaundice until today. Phenobarbital is an anti-epileptic agent used for treatment of prolonged jaundice.[6] Furthermore, phenobarbital is not routinely used for treatment of neonatal unconjugated

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hyperbilirubinemia due to different causes.[7] It has adverse effect potential such as sedation and neurological development abnormalities.[8] Furthermore, experimental studies showed that phenobarbital reduces oxidative metabolism of unconjugated bilirubin (UCB) and may cause an increase in the neurotoxicity in the rat brain.[9] Ursodeoxycholic acid (UDCA) is widely used for the treatment of cholestatic liver diseases and well tolerated in pediatric patients.[10] In addition to phototherapy used in the treatment of indirect bilirubinemia in recent years, studies related to ursodeoxycholic acid (UDCA) as an adjuvant treatment are interesting. When the common aims and results of the studies are examined, it is very important that UDCA shortens the duration of phototherapy and hospitalization in most of them. In this way, the risks that the newborn baby will face during his stay in the hospital are reduced and the time for the mother and baby to meet is shortened.[11-13] Gourley *et al*. showed that infants fed formulas containing hydrolyzed casein that inhibit beta glucuronidase had lower levels of jaundice than those fed routine formula or breast milk. Of course, more extensive studies are needed for this evidence.[14] In another study of Gourley *et al*., this time L-aspartic acid was shown to be effective in reducing jaundice. However, whey/casein, which does not inhibit beta glucuronidase, also plays a role in reducing jaundice.[15] That is why studies are continuing to reduce jaundice through different mechanisms in patients with prolonged jaundice. From this point of view, we focused on UDCA. UDCA is a bile acid and prevents reabsorption of bilirubin from the intestines and also well tolerated.[16,17] UDCA helps in improving endogenous bile secretion, reducing enterohepatic circulation and displacement of more toxic components of endogenous bile acids. UDCA also exerts neuroprotective and hepatoprotective properties through its anti-inflammatory, anti-apoptotic, and antioxidant effects.[18,19] Therefore, we believe that UDCA has a promising effect in order to contribute to the current literature seeking an alternative to other drugs in the treatment of prolonged jaundice with a superiority for adverse effects. Honar *et al*. showed in their study conducted to investigate the combination of phototherapy and UDCA in hospitalized patients with unconjugated hyperbilirubinemia that the initiation of UDCA immediately after hospitalization or within the first 48 hours reduced the length of hospital stay by presenting an additive effect with phototherapy.[20] El-Gendy *et al*. obtained similar outcomes in their study.[21] In the study where different doses of UDCA were tried in neonatal jaundice; Gharehbaghi *et al*. detected that UDCA dose of 7.5 mg/kg significantly reduced mean phototherapy duration and mean hospitalization duration when compared with 5 mg/kg.[22] In another randomized controlled study, UDCA doses of 10 mg/kg/day and 20 mg/kg/day were compared; it was concluded that hyperbilirubinemia would recover faster with phototherapy and 10 mg/kg/day UDCA.[23] In their meta-analysis, Kuitunen *et al.* reviewed six studies involving 880 newborns investigating the relationship between neonatal hyperbilirubinemia and UDCA. They concluded that UDCA shortened the duration of phototherapy by approximately 20 hours, thus a clinically relevant finding that would benefit patients and families.[24] In the meta-analysis, results evaluating the use of UDCA in indirect hyperbilirubinemia used 10 mg/kg twice daily.[24] This study was planned to investigate the use of UDCA and evaluate the efficacy in the treatment of patients with prolonged unconjugated hyperbilirubinemia who are breastfed without any etiological factors.

Materials and Methods

Study population

This study is a randomized controlled double-blind study. The study included 47 term newborns who were referred to neonatology outpatient clinic of Erciyes University Medical Faculty because of prolonged jaundice. Patients were included in the study completely randomly and with double-blinding and allocation concealment. Power analysis of the G*Power 3.1.9.4 for Windows package program was performed. The number of patients and controls was determined with 95% power and 5% error conditions. The criterion for prolonged jaundice was accepted as total bilirubin level over 10 mg/dl in a term newborn after 14 days of delivery.[2,25] Parents who have not agreed to participate in the study, those who have not used the treatment regularly, have not attended the control visits regularly premature babies and patients who received another treatment (hypothyroidism, urinary tract infection, and hemolytic anemia) due to the etiological factor causing prolonged jaundice were excluded from the study. In addition, newborns with direct bilirubin levels above 1 mg/dl were excluded from the study. The study was approved by Ethical Committee of Medical Faculty of Erciyes University, and written consents of parents were obtained. Detailed prenatal, natal, and postnatal histories were obtained from the parents of babies with prolonged jaundice, and physical examinations were performed. Blood types of mothers and infants, postnatal age, previous history of phototherapy or blood exchange, breastfeeding status of the infants, and number of defecations of infants were recorded in the postnatal history.

Sampling and test study method

Complete blood count, peripheral blood smear analysis, reticulocyte count, blood types of mothers and infants, direct Coombs test, total and direct bilirubin, serum aspartate transaminase (AST), serum alanine transaminase (ALT), viral infection markers, thyroid function tests, glucose-6-phosphate dehydrogenase enzyme level, urine analysis, urine culture, and C-reactive protein for sepsis evaluation were ordered from patients with total serum bilirubin level over 10 mg/dl. Furthermore, abdominal ultrasound, tandem mass, and reducing substance as well as metabolic screening in the urine were planned for patients whose clinical finding and laboratory tests required or those with direct bilirubin elevation. Patients with lower free serum T4 level and higher TSH level were diagnosed with congenital hypothyroidism. Cause-oriented treatment was started for infants with detected etiology; these patients were excluded from the study. The infants who have been breastfed without any known etiology were followed with the diagnosis of breastfeeding-induced prolonged jaundice, and enrolled in the study. Newborns diagnosed with prolonged jaundice with a bilirubin level above 10 mg/dl were followed in the outpatient clinic; 10 mg/kg/day of UDCA was administrated divided in two doses for 7 days. The control group was also given a placebo. The control group was also given a placebo. In the outpatient clinic, both the researcher and the patient's relatives did not know which group they belonged to. Bilirubin levels, body weight, and liver enzyme levels were measured on days 4 and 7 after the treatment; a number of defecations were followed. Patients with prolonged indirect hyperbilirubinemia were evaluated for bilirubin reduction rate, side effects, and duration of jaundice one week after UDCA treatment. Participants were evaluated for bilirubin decrease rate, adverse effect, and duration of jaundice. Infants whose bilirubin level decreased below 10 mg/dl during follow-up were excluded from the monitoring.

Statistical analysis

Statistical analyses were performed by SPSS ver 22.0. Shapiro–Wilk test was used to review whether data distributed normally; values were expressed in mean ± standard deviation. In comparison of the data of study and control groups, independent samples *t* test was used if the data of both groups were normally distributed, and the Mann–Whitney U test was used if the data of at least one group were not normally distributed. Total bilirubin and direct bilirubin levels were compared at the time of admission and on the 7th and 14th days of UDCA treatment. If differences between measurements distribute normally, Repeated-measures one-way ANOVA test was used; and if at least one difference does not distribute normally, Friedman test was used. Differences between before and after values were calculated to make a comparison between the before and after values of ALT, AST, and gamma glutamyl transferase (GGT) measurements in both groups. Any *P* value below 0.05 ($P < 0.05$) was accepted as statistically significant. The ROC analysis was performed through SPSS ver 22.0 and easyROC: a web-tool for ROC curve analysis (ver. 1.3).[26] Youden cutoff method was used to determine cutoff values.

Two patients evaluated within the scope of the study were excluded from the study because they have not used the drug regularly; three patients were excluded from the study because of ignoring the control visits. Considering the etiological reasons, eight patients were excluded because of blood incompatibility, one patient was excluded because of congenital hypothyroidism, and three patients were excluded because of treatment for urinary tract infection. Forty-seven patients who were breastfed and had no underlying etiological cause were enrolled; 27 patients were randomly included in the study group, and 20 patients were included in the control group.

Results

Demographic characteristics of cases are summarized in Table 1. Bilirubin levels, liver enzymes, daily defecation count, and hemogram parameters of patients with prolonged jaundice who have/have not received UDCA treatment were compared at referral and during follow-ups. Total and direct bilirubin levels were similar in both groups at referral ($P = 0.84$; $P = 0.77$); however, total bilirubin level of the study group receiving UDCA therapy was significantly lower at days 7 and 14 ($P < 0.001$) [Table 2].

Comparison of total bilirubin levels in patients receiving UDCA treatment depending on the days revealed that there was a statistically significant difference between levels before the treatment, levels at days 4 to 7, and day 14 ($P < 0.001$); however, a significant difference was detected in levels before the treatment and at day 14 ($P = 0.002$) [Table 3]. The number of defecations per day was significantly higher in the group receiving UDCA treatment compared to those not receiving UDCA ($P = 0.03$) [Table 1]; a statistically significant increase was found after the treatment compared to before ($P < 0.001$) [Table 2]. Total bilirubin levels gradually decreased in days in the groups treated and not treated by UDCA ($P < 0.001$ vs $P = 0.002$); and such decrease was statistically significant in the UDCA treatment group at days 7 and 14 ($P < 0.001$) [Table 3].

Table 1: Comparison of parameters in patients with prolonged jaundice receiving and not receiving UDCA treatment

Variable	Prolonged jaundice		*P*
	UDCA (+) (*n*=27)	UDCA (-) (*n*=20)	
Body weight (g)	3611.85±591.51	3910.50±247.88	0.02
Postnatal age (days)	25.44±6.39	22.25±4.90	0.06
At referral			
Daily defecation count	4.81±1.24	4.05±1.09	0.03
Hemoglobin (gr/dl)	13.11±1.81	14.49±1.66	0.07
White blood cell (10^3/µl)	8971.11±2479.83	9521.00±1735.22	0.37
Platelet count (10^3/µl)	239074.07±102962.62	239400.00±46744.99	0.99
Reticulocyte count (10^3/µl)	1.12±0.71	1.09±0.30	0.08
AST (U/L)	35.29±13.31	35.40±12.11	0.97
ALT (U/L)	20.00±7.61	21.70±12.50	0.56
GGT (U/L)	99.77±16.07	101.55±18.00	0.09
Total Bilirubin (mg/dL)	16.02±1.41	15.93±1.66	0.84
Direct Bilirubin (mg/dL)	0.69±0.20	0.67±0.34	0.77
At control (day 7)			
Daily defecation count	6.14±1.61	4.10±0.96	<0.001
AST (U/L)	26.14±6.12	33.10±12.47	0.03
ALT (U/L)	15.74±5.66	18.80±7.09	0.10
GGT (U/L)	88.66±17.03	124.85±39.24	0.001
UDCA Post-treatment			
Day 7 T. Bilirubin (mg/dL)	8.18±2.31	13.92±2.66	<0.001
Day 7 D. Bilirubin (mg/dL)	0.51±0.15	0.63±0.25	0.09
Day 14 T. Bilirubin (mg/dL)	5.45±2.59	11.91±2.83	<0.001
Day 14 D. Bilirubin (mg/dL)	0.38±0.18	0.54±0.19	0.006

AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, GGT=Gamma glutamyl transferase, T. Bilirubin=Total Bilirubin, D. Bilirubin=Direct Bilirubin, UDCA=Ursodeoxycholic acid. Data were expressed as mean±standard deviation

Table 2: Comparison of parameters before and 7 days after UDCA treatment in patients with prolonged jaundice

Variable	UDCA treatment		Δ	*P*
	Before (*n*=27)	After (*n*=27)		
Number of defecation	4.81±1.24	6.14±1.61	5.14±1.61	0.001
Hemoglobin (gr/dl)	13.11±1.81	12.51±1.65	11.51±1.65	0.01
White blood cell (10^3/µl)	8971.11±2479.83	8270.00±1229.69	8269.00±1229.69	0.07
Platelet count (10^3/µl)	239074.07±102962.62	229851.85±71051.28	229850.85±71051.28	0.46
AST (U/L)	35.29±13.31	26.14±6.12	25.14±6.12	0.002
ALT (U/L)	20.00±7.61	15.74±5.66	14.74±5.66	0.003
GGT (U/L)	99.77±16.07	88.66±17.03	87.66±17.03	0.001

AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, GGT=Gamma glutamyl transferase, UDCA=Ursodeoxycholic acid. Data were expressed as mean±standard deviation. Δ = (After–Before)/Before

Since the control group has no bilirubin level on day 4, bilirubin levels at day 4 could not be compared. Evaluation of defecation counts were 4.81 ± 1.24 and 4.05 ± 1.09 in UDCA treatment group and the control group, respectively (P = 0.03). It was detected that the number of defecations increased significantly in the UDCA treatment group when compared to the control group (6.14 ± 1.61 vs 4.10 ± 0.96) (P < 0.001). No adverse effect was detected in any case.

The optimal cutoff value of AST levels after UDCA treatment due to prolonged jaundice was found 21 U/L with specificity of 88.9%, sensitivity of 45%, and area under the curve 0.666 (95% CI: 0.500-0.833). The AST <21 (U/L) level after UDCA treatment is considered that the treatment is effective in patients with prolonged jaundice. It was found that the decrease in AST values after the treatment in patients who received UDCA treatment due to prolonged jaundice was a stimulating factor for

Table 3: Comparison of total and direct bilirubin levels in patients with prolonged jaundice including those receiving UDCA treatment and the control group

Variable	Before the treatment	After UDCA treatment			*P*
		Day 4	Day 7	Day 14	
UDCA (+) (*n*=27)					
T. Bilirubin (mg/dL)	16.02±1.41[a]	11.52±2.12[b]	8.18±2.31[c]	5.45±2.59[d]	<0.001
D. Bilirubin (mg/dL)	0.69±0.20[a]	0.58±1.61[a]	0.51±0.15[b]	0.38±0.18[c]	<0.001
UDCA (-) (*n*=20)					
T. Bilirubin (mg/dL)	15.93±1.66[a]	-	13.92±2.66[a]	11.91±2.83[b]	0.002
D. Bilirubin (mg/dL)	0.67±034	-	0.63±0.25	0.54±0.19	0.10

T. Bilirubin=Total Bilirubin, D. Bilirubin=Direct Bilirubin, UDCA=Ursodeoxycholic ACID. Data were expressed as mean±standard deviation. Different letters included in the same line express the difference, and same letters express the similarity

Table 4: Predictive value of liver function tests in patients with prolonged jaundice before and 7 days after UDCA treatment

Groups (Pre–Post treatment)		Area under the curve		Diagnostic measures					
		AUC	*P*	SEN (95%CI)	SPE (95%CI)	PPV	NPV	LR+	LR-
AST (U/L)	Previous AST (>25)	0.519 (0.39-0.689)	0.822	0.900 (0.683-0.988)	0.222 (0,086-0.423)	0.462 (0,221-0.884)	0.750 (0.418-0.885)	1.157 (0.902-1.484)	0.450 (0.101-2.001)
ALT (U/L)	Further AST (<21)	0.666 (0,500-0.833)	0.049	0.450 (0,231-0.685)	0.889 (0,708-0.976)	0.750 (0.477-0.888)	0.686 (0.444-0.919)	4.050 (1.255-13.072)	0.619 (0.407-0.940)
	Previous AST (>31)	0.488 (0.313-0.664)	0.901	0.200 (0,057-0.437)	0.926 (0,757-0.991)	0.667 (0.333-0.861)	0.610 (0.275-0.932)	2.700 (0.547-13.318)	0.864 (0.677-1.102)
	Further AST (<26)	0.609 (0,442-0.775)	0.198	0.250 (0,087-0.491)	0.600 (0,361-0.809)	0.579 (0.341-0.759)	0.429 (0.239-0.679)	1.019 (0.504-2.058)	0.988 (0.614-1.589)

AST=Aspartate aminotransferase, ALT=Alanine amino transferase, AUC=Area under the curve, SEN=Sensitivity, SPE=Specificity, PPV=Positive predictive value, NPV=Negative predictive value, LR+ = Positive likelihood ratio, LR- = Negative likelihood ratio

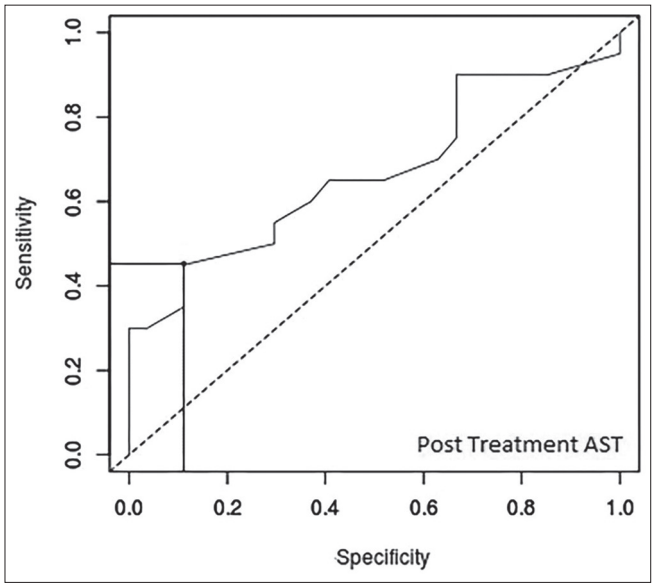

Figure 1: The ROC curve of post-treatment AST level in patients with prolonged jaundice receiving UDCA therapy

the effect of UDCA treatment (P = 0.04). Diagnostic measurements about AST and ALT are provided in Table 4. The ROC curve of AST levels in patients who developed prolonged jaundice in those treated with UDCA and in the control group is shown in Figure 1.

Discussion

The findings of our study showed that UDCA rapidly reduced UCB in patients with prolonged jaundice. There are many clinical and laboratory studies on the pharmacological treatment of unconjugated hyperbilirubinemia in the literature. Pharmacological agents used in these studies are effective in different stages of bilirubin metabolism, and metalloporphyrins that inhibit hem oxygenase competitively have been emphasized.[27,28] However, routine use is not recommended despite being a promising medical treatment. On the other hand, the most commonly used pharmacological agent for treatment of prolonged jaundice is phenobarbital.[6] It was shown that administration of phenobarbital to the mother before birth and to the baby after birth reduces the development of severe hyperbilirubinemia and the need for exchange transfusion.[8] However, adverse effects limit use of this drug.

In fact, most of the newborns with prolonged jaundice do not have any underlying pathology and are defined as breast milk jaundice.[3] However, polymorphisms in the promoter region of the UGT1A1 gene and mutations in the exon region are held responsible for most of these

cases.[29] We detected in our study that UDCA rapidly reduced UCB in patients with prolonged jaundice through same mechanism. We thereby believe that outpatient clinic referral of patients may decrease.

Bile salts form the major organic components of saffron. Bile salt administration reduces plasma UCB concentration through several mechanisms. First mechanism of bile salt administration is increasing biliary excretion of organic anions including UCB; second mechanism is the slight decrease in fat absorption in healthy volunteers treated by UDCA. This situation is thought to be similar to the hypobilirubinemic effect caused by orlistat used in rats and Crigler-Najjar patients.[30-32] Nishioka *et al.* demonstrated that orlistat, a lipase inhibitor, increases fecal excretion of both UCB and fat.[33] Therefore, bile salt administration binds the UCB in the intestinal lumen and increases the fecal excretion of bilirubin. While planning our study, we planned to evaluate serum lipid levels along with the decrease in bilirubin level. However, since the evaluation of the eighth day coincided with a holiday in some of the cases, measurement could not be made due to laboratory working conditions. It was not studied because there were not enough test measurements to be evaluated in the study group. Furthermore, we could not measure fecal fat excretion. Therefore, the exact role of UDCA on fat absorption reducing effect to reduce UCB could not be evaluated thoroughly.

On the other hand, Cuperus *et al*. showed in their study on Gunn rats that the plasma UCB concentration decreased significantly with addition of ursodeoxycholic acid or colic acid to the diet. In the study mentioned above, 300 mg/kg/day UDCA dose was administered; a decrease was detected within 3 days and a maximum decrease by 40% was detected within two weeks.[34] In the present study, 10 mg/kg/day was administrated to newborns dividend in two doses. Treatment period was determined as 7 days by considering the bilirubin reduction rate and compliance of parents. In our study, the rate of bilirubin decrease was evaluated on the fourth and seventh days after initiation of the treatment. On the fourth day, there was a decrease of 30% on the fourth day and by 50% on the seventh day when compared with initial total bilirubin. Furthermore, it was observed that the decrease continued in the control exam performed one week after the treatment was discontinued in our study.

UDCA is well tolerated in pediatric patients and commonly used for the treatment of cholestatic liver disease.[10] Some studies have suggested an association between plasma bilirubin levels and gastrointestinal transit. Conditions that slow/delay the gastrointestinal transit such as malnutrition or Hirschsprung's disease have been associated with chronic neonatal jaundice.[35-37] It was remarkably suggested that oral treatments as well as phototherapy accelerate the gastrointestinal transit. Phototherapy and intense phototherapy in particular may cause diarrhea.[38] We found that the defecation count increased in a statistically significant manner when we compared the number of stools before starting UDCA treatment and during treatment. However, such increase was an increase in defecation count rather than diarrhea. Therefore, the increase in the defecations is a side effect and contributes to the hypobilirubinemic effect.

This study is the first study investigating the efficiency of UDCA treatment in newborn infants with prolonged unconjugated hyperbilirubinemia; outcomes are important due to superiority to other medical treatments with more adverse effect potential. In some cases of prolonged jaundice, liver function tests, especially AST, are slightly elevated. CR Winfield and R MacFaul found mild elevation of AST in four of the patients in their 12 case series of prolonged jaundice.[39] In addition, the effect of UDCA on AST is not clearly known. We think that UDCA provides a decrease in AST due to its antioxidant effect. Although the decrease in AST levels by UDCA treatment is significant, lower sensitivity may be explained by limited sample size. Therefore, further studies with higher sensitivity and specificity are needed. Furthermore, we could not examine all important variables due to the lack of similar studies on the effect of UDCA in the treatment of prolonged jaundice. Other limitations of the study are lack of multi-centered experiences and lack of long-term follow-ups.

Conclusions

Consequently, we believe that UDCA may be a promising pharmacological agent in the management of prolonged unconjugated hyperbilirubinemia with this study. It was observed in the present study that UDCA is an effective oral therapy to reduce unconjugated bilirubin in newborns with prolonged jaundice. Furthermore, the AST <21 (U/L) level after UDCA treatment stressed out as an effective treatment in patients with prolonged jaundice. Therefore, AST decrease may be used to evaluate the treatment efficiency. But it is appropriate to be recommended not in every breast milk jaundice, but in selected cases where the jaundice does not decrease or the process is prolonged.

Acknowledgement

Research team members and all the mothers who consented to the study.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval was obtained from the local ethics committee (Erciyes Medical Faculty). Written consent was obtained from the participants enrolled in the study.

Consent for publication

Not applicable.

Abbreviations

UDCA = Ursodeoxycholic acid, AST = Serum aspartate aminotransferase, ALT = Serum alanine transaminase, GGT = Gamma Glutamyl Transferase, UCB = Unconjugated bilirubin

Authors' contributions

AO, SK, HH, and OB conceptualized the study. AO and OB conducted the first draft of the analysis. SK and HH reviewed the statistical analysis. AO and SK made the first draft. AO, SK, HH, and OB reviewed and revised the manuscript. All authors read and approved the final version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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