Original Article

J Babol Univ Med Sci
23; 2021. P: 70-75

Comparison of the Effect of Phototherapy with Oral Calcium Versus Phototherapy Alone in the Treatment of Unconjugated Hyperbilirubinemia in Healthy Term Infants

M. Habibi (MD)[1], A. Karbord (MSc)[2], M. Vahidi (MD)[3], F. Samiee Rad (MD)*[4]

1.Department of Pediatric, School of Medicine, Qazvin University of Medical Sciences, Qazvin, I.R.Iran

2.Department of Operating Room, School of School of Allied Medical Sciences, Qazvin University of Medical Sciences, Qazvin, I.R.Iran

3.School of Medicine, Qazvin University of Medical Sciences, Qazvin, I.R.Iran

4.Department of Pathology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, I.R.Iran

J Babol Univ Med Sci; 23; 2021; PP: 70-75

Received: Aug 4th 2020, Revised: Sep 30th 2020, Accepted: Dec 22nd 2020.

ABSTRACT

BACKGROUND AND OBJECTIVE: Jaundice (hyperbilirubinemia) is one of the most common clinical complaints during infancy among term and premature infants during the first week of life. Phototherapy is an effective and accepted treatment for neonatal hyperbilirubinemia, which may be associated with complications such as skin, eye and electrolyte disorders. This study was performed to compare the effect of phototherapy with oral calcium versus phototherapy alone in the treatment of unconjugated hyperbilirubinemia in healthy term infants.

METHODS: This clinical trial study was performed on 50 healthy term infants with jaundice (serum bilirubin 6.9-21 mg/dL). Neonates were randomly divided into intervention (50 mg/kg body weight of oral calcium with phototherapy) and control (phototherapy) groups. Data related to age, gender, birth weight, gestational age, number of hospitalization days and bilirubin level at the beginning of hospitalization and at 24, 48 and 72 hours were collected and compared in a checklist.

FINDINGS: Decrease in total bilirubin level was observed with a significant difference between the two groups (p=0.000). The mean unconjugated hyperbilirubinemia showed significant difference in the intervention group (2.1±0.5 mg/dl) and the control group (2.6±1.3 mg/dl) (p=0.03). The changes in unconjugated hyperbilirubinemia in repeated measures was also significant in the intervention group (p=0.01).

CONCLUSION: The results of the study showed that oral calcium with phototherapy may be effective in reducing neonatal jaundice.

KEY WORDS: *Infant, Unconjugated Hyperbilirubinemia, Phototherapy, Calcium, Oral, Treatment.*

Please cite this article as follows:

Habibi M, Karbord A, Vahidi M, Samiee Rad F. Comparison of the Effect of Phototherapy with Oral Calcium Versus Phototherapy Alone in the Treatment of Unconjugated Hyperbilirubinemia in Healthy Term Infants. J Babol Univ Med Sci. 2021; 23: 70-5.

***Corresponding Author: F. Samiee Rad (MD)**
Address: Kosar Hospital, Qazvin University of Medical Sciences, Qazvin, I.R.Iran
Tel: +98 28 33236375
E-mail: fsamieerad@gmail.com

Introduction

Jaundice (hyperbilirubinemia) is one of the most common clinical complaints during infancy among term and premature infants during the first week of life (1, 2). Probably the main mechanism of its creation is the imbalance between production and excretion of bilirubin (3). Most infants go through this stage with supportive treatment and the rest with high-powered phototherapy or blood transfusions (4).

Phototherapy is an effective and accepted treatment for neonatal hyperbilirubinemia. Phototherapy may cause side effects such as dehydration, skin lesions, hyperthermia, eye damage (toxic effects on the retina), corneal scar, conjunctivitis, oxidative DNA damage, diarrhea, electrolyte disturbances, central nervous system complications, hypocalcemia, etc. in the infant (5, 6). Therefore, several studies have been performed in recent years on the use of different methods and treatments using various substances and with fewer complications. Among these studies, we can mention the effect of chicory extract, probiotics and oral zinc sulfate in accelerating and improving the treatment process of hyperbilirubinemia. However, the safety and efficiency of these methods as alternatives to the phototherapy method require extensive studies with a large sample size (7, 8).

Calcium homeostasis is an interesting mechanism in infancy. At birth, umbilical cord blood has higher calcium levels than the mother's blood. In the first few days of a normal infant's life, blood calcium levels progressively decrease and reach a minimum level on the second and third days of life. In term infants, blood calcium levels return to normal levels on the tenth day after birth (9-11). Phototherapy increases calcium absorption from bone and decreases melatonin levels. Changes in melatonin levels cause phototherapy-induced hypocalcemia. Studies by Asl et al. and Bhat et al. showed that one of the causes of hypocalcemia was decreased activity of parathyroid hormone. On the other hand, urinary excretion of calcium (hypercalciuria) was higher in infants undergoing phototherapy (11, 12).

Unconjugated hyperbilirubinemia in the intestine is made by the enzyme β-glucuronidase and reabsorbed through the enterohepatic cycle. Interference of amorphous calcium phosphate in the enterohepatic cycle reduces the serum level of unconjugated hyperbilirubinemia. Although human studies are very limited in this area, but in a study in rats, the effect of oral treatment with calcium phosphate on reducing unconjugated hyperbilirubinemia and increasing its excretion in the feces has been shown (13, 14). Furthermore, studies on patients with Crigler–Najjar syndrome (inherited unconjugated hyperbilirubinemia) type 1 showed that receiving calcium supplements, while receiving routine phototherapy, reduces the serum level of unconjugated bilirubin. The mechanism of action was interference in the enterohepatic cycle, binding of calcium to bilirubin in the gastrointestinal tract and increasing its excretion in the feces (14, 15). Side effects of calcium phosphate treatment are very rare. In common therapeutic interventions, side effects have been reported in humans and animals. Rare cases of possible calcium deposition in the kidney have been reported in chronic use of the drug (16).

Based on the above evidence on the effect of oral calcium in improving and accelerating the treatment of unconjugated hyperbilirubinemia, the high prevalence of neonatal jaundice, its significant complications, hospitalization costs, hospital care, complications and subsequent nosocomial infections, and necessity of taking measures to reduce the irreversible side effects, the need for interventions to replace the conventional and powerful phototherapy with methods that are less invasive, simple, safe, effective and inexpensive is felt more than ever. Regarding the use of oral supplements, some studies have been performed about oral zinc supplements.

Considering the performed investigations, no comprehensive and experimental study has been conducted so far on the role of oral calcium supplements in the treatment of unconjugated hyperbilirubinemia. Therefore, this study was performed to determine the effects of oral calcium combined with phototherapy in the treatment of unconjugated hyperbilirubinemia.

Methods

This study is a double-blind randomized clinical trial with registration number IRCT2017070534907N1 in the clinical trial system and ethics code IR.QUMS.REC.1396.125 from the ethics committee of Qazvin University of Medical Sciences. It was conducted among infants (37-40 weeks) with jaundice who were hospitalized due to unconjugated hyperbilirubinemia in the neonatal ward of Kowsar Teaching Hospital (level three neonatal care). Term infants with physiological jaundice, birth weight 2500-4000 g, age 2-7 days, exclusive breastfeeding, serum bilirubin level 6.9-21 mg/dL less than 15% of which

was direct bilirubin (direct serum bilirubin level in these infants was less than 2 mg/dL) were included in the study. Infants with signs of jaundice in the first 24 hours after birth, preterm and post-term infants (under 36 weeks and over 40 weeks), infants with Intrauterine Growth Restriction (IUGR), HDN caused by ABO incompatibility, Rh incompatibility, glucose-6-phosphate dehydrogenase deficiency (G6PDD) or any other known cause of jaundice in the infant, congenital anomalies, systemic and infectious diseases, and infants taking any medication or supplement by any means were excluded. All infants were treated for jaundice based on the gold standard of treatment (17) and the appropriate protocol during two months in 2018 and were not prohibited from any standard treatment.

Then, after providing full explanations by the researcher, written informed consent was obtained from all parents of the infants. Fifty term neonates with jaundice were randomly divided into two groups of intervention (25 patients) and control (25 patients). To randomize the study, the block randomization method was used so that parents and evaluators were unaware of random allocation.

Hospitalization criteria were serum bilirubin above 12 mg/dL and discharge criteria were less than 10 mg/dL (in hemolytic jaundice) and less than 12 mg/dL (in non-hemolytic jaundice). Serum bilirubin levels were measured in both groups at the beginning of hospitalization and then at 24 and 48 hours (at intervals of 8-12 hours in hemolytic type and every 24 hours in non-hemolytic type). If hospitalization continued and jaundice persisted in the infant, bilirubin levels were measured 72 hours after hospitalization.

Assessments were performed in accordance with the routine care and in accordance with standard diagnostic and treatment guidelines. Evaluations were performed to observe the reduction of the serum levels of total bilirubin and indirect bilirubin based on history and examinations in order to confirm the result of using phototherapy combined with oral calcium. Infants in both groups were treated with high-powered phototherapy according to the standard treatment instructions (18) using a 12-lamp phototherapy device made by Tosan, Iran. The intervention group received calcium gluconate syrup, under the brand name Calciram (Ramo Farmin Co, made in Iran) at a dose of 2 mg/kg body weight (2 cc) every 12 hours until the jaundice resolved (19).

The drug was given to the intervention group by the pediatrician and the nurse in charge of the ward. If infants vomited during treatment up to two hours after taking the drug, calcium therapy was stopped and the patient was excluded from the trial. In the control group, all neonates were given distilled water with a volume of 2 cc, similar to the treatment group, as a placebo. In these infants, in case of vomiting, the placebo treatment was stopped and the patients were excluded from the study. The primary and secondary outcomes of using phototherapy combined with oral calcium on the reduction of serum levels of total bilirubin and indirect bilirubin were investigated.

For laboratory evaluation of total, indirect and direct bilirubin levels, one cc of blood was collected in a test tube without anticoagulant by trained personnel. Then the serum was separated. For photometric analysis, calibrated Selectra 2 autoanalyzer and Pars Azmoon kit were used. Bilirubin levels were reported based on dl/mg. Data about age, gender, birth weight, gestational age, number of hospitalization days, and bilirubin levels of these infants at different hours were collected in a checklist. Then the data were entered into SPSS 22 software and analyzed by calculating the absolute frequency and percentage, mean and standard deviation, t-test, and the repeated measures ANOVA. $p<0.05$ was considered significant.

Results

In the calcium treatment group, 52% were male and 48% were female infants and in the control group, 56% were male and 44% were female infants and were almost identical in terms of gender. In the intervention group, the mean age of the neonates was 3.1±1.7 days and in the placebo group, it was 2.9±1.7 days and they were similar in the number of hospitalization days (Table 1).

The decreasing trend of changes in total bilirubin in 24 hours ($p=0.048$) and 48 hours ($p=0.027$) after hospitalization between the intervention and control groups was statistically significant. In the final evaluation, there was a significant decrease in total bilirubin between the intervention and control groups in the beginning of hospitalization compared to 24 and 48 hours later ($p=0.006$) (Table 2).

The decreasing trend of changes in indirect bilirubin in 24 hours ($p=0.02$) and 48 hours ($p=0.04$) after hospitalization between the intervention and control groups was statistically significant. In the final evaluation, there was a significant decrease in indirect bilirubin between the intervention and control groups in the beginning of hospitalization compared to 24 and 48 hours later ($p=0.00$) (Table 3).

Table 1. Demographic characteristics of neonates in the intervention and control groups

Demographic information	Intervention group Mean±SD Number(%)	Control group Mean±SD Number(%)
Age at hospitalization (days)	3.1±1.7	2.9±1.7
Gestational age	39.9±1.2	38.6±1.1
Number of hospitalization days	1.8±0.6	1.6±0.8
Birth weight	3198.4±669.7	3236.4±348.2
Gender		
Boy	13(0.52)	14(0.56)
Girl	12(0.48)	11(0.44)

Table 2. Total bilirubin (mg/dL) in neonates participating in the study

Bilirubin level	Intervention group Mean±SD	Control group Mean±SD	p-value
Total bilirubin level at hospitalization	15.9±4.1	16.7±6.6	0.031
Total bilirubin level 24 hours after hospitalization	13.7±2.2	14.8±1.8	0.048
Total bilirubin level 48 hours after hospitalization	11.8±3.8	13.7±2.7	0.027
Total bilirubin in the final assessment	10.1±1.3	12.4±2.4	0.001
Total p-value	0.006		

Table 3. Direct bilirubin (mg/dL) in the study groups

Bilirubin level	Intervention group Mean±SD	Control group Mean±SD	p-value
Direct bilirubin level at hospitalization	0.58±0.32	0.66±0.28	0.436
Direct bilirubin level 24 hours after hospitalization	0.53±0.14	0.60±0.45	0.02
Direct bilirubin level 48 hours after hospitalization	0.37±0.11	0.51±0.37	0.04
Direct bilirubin in the final assessment	0.39±0.17	0.49±0.13	***0.004
Total p-value	**0.000		

Discussion

In this study, the decreasing trend of total and indirect bilirubin in 24 hours and 48 hours after hospitalization between the intervention and control groups was statistically significant. These results indicate the significant effect of oral calcium combined with phototherapy in reducing total and direct bilirubin in infants who are healthy and only have jaundice. In the final evaluation, there was a significant decrease in total and indirect bilirubin between the intervention and control groups in the beginning of hospitalization compared to 24 and 48 hours later. This can reduce the length of hospital stay. A study by Olusanya et al. found that factors such as low gestational age at birth, hematoma and bruises, or exclusive breastfeeding, which may cause insufficient calcium intake and are associated with weight loss, can still reduce severe hyperbilirubinemia. Risk factors for this complication include low age, low weight, hemolytic anemia and sepsis (19). Conjugated bilirubin is converted to unconjugated bilirubin in the intestine by the enzyme β-glucuronidase. This compound has two destinies: either it is converted to urobilinoid under the influence of the intestinal flora or is reabsorbed through the enterohepatic cycle. Therefore, therapies for reducing unconjugated bilirubin are focused on interventions in the enterohepatic cycle. Studies have shown that amorphous calcium phosphate has a strong tendency to bind to unconjugated bilirubin and its sediment. In the dose-dependent enterohepatic cycle, this intervention occurs only through insoluble amorphous calcium phosphate and not the ionized calcium. Human research on the use of this treatment is rare. The results of the investigations by researchers showed the effect of oral treatment with calcium phosphate on decreased serum unconjugated bilirubin levels through its deposition in the intestine and thus its increased excretion in the feces (13, 14, 20). On the other hand, phototherapy inhibits the pineal gland, reduces melatonin levels and ultimately causes hypocalcemia. Melatonin also plays an important role in blocking the effect of cortisol on calcium absorption in bone. Therefore, in melatonin deficiency, more calcium is absorbed by the bones through cortisol. Furthermore, urinary excretion of calcium (hypercalciuria) with be more in infants under phototherapy. As a result, hypocalcemia due to phototherapy reduces the binding of calcium to

unconjugated bilirubin in the intestinal lumen and reduces its excretion through the feces. As a result, unconjugated bilirubin is reabsorbed from the enterohepatic cycle and enters the bloodstream (11, 12). Although there is ample evidence of a link between normal blood calcium levels and improved treatment of unconjugated hyperbilirubinemia in various articles (11, 12, 20), very few genuine studies have been performed to accelerate the effect of oral calcium in the treatment of unconjugated hyperbilirubinemia.

A study by Hafkamp et al., which examined oral treatment of unconjugated hyperbilirubinemia in rats, showed that oral treatment with calcium phosphate supplements reduced plasma levels of unconjugated bilirubin. This study showed that single-drug oral therapy was as effective as the use of phototherapy in these mice. Furthermore, combination therapy with calcium phosphate was more effective than phototherapy alone. The most important mechanism of action was the enterohepatic cycle and lack of reabsorption of unconjugated bilirubin (21).

The main difference between the study of Hafkamp et al. and the present study is related to the study population. Therefore, generalization of results to humans should be done with caution. The results of the study by Van der Veere et al. aimed at investigating the effect of oral calcium phosphate supplements on serum levels of unconjugated bilirubin in patients with Crigler-Najjar syndrome type 1 showed that after three weeks of simultaneous phototherapy and taking oral calcium phosphate supplements, the serum level of unconjugated bilirubin decreased. Side effects of oral supplements were minimal. Therefore, they recommended the use of this oral supplement along with conventional phototherapy treatment (22). The main difference between the study of Van der Veere et al. and the present study is the study population; their study included adults with Crigler-Najjar syndrome type 1 syndrome, while the study population in the present study included term infants with jaundice. As a result, interpreting and generalizing their results to infants requires careful consideration of different physiological conditions of the infant.

Significant reduction in the total and direct bilirubin in calcium and phototherapy group compared to the control group may indicate the positive effect of oral calcium intake in the treatment of neonatal jaundice. Moreover, further reduction of serum levels of this substance in the calcium and phototherapy group compared to phototherapy alone may indicate the efficacy of this treatment. It is suggested that studies with larger sample size be performed in this regard in the future.

Conflict of interest: All authors state that they have no conflict of interest in this study.

Acknowledgment

We would like to thank the Vice Chancellor for Research and Technology of Qazvin University of Medical Sciences for supporting this research, as well as Ms. Tofighi, staff member in the neonatal ward, Mr. Toghianifar, the laboratory expert, Research Development Unit of Kowsar Hospital, especially Ms. Zahra Sadat Mohammadi who assisted us in analyzing the data and also Ms. Samin Dokht Molaverdi Khani.

References

1.Boskabadi H, Sezavar M, Zakerihamidi M. Evaluation of neonatal jaundice based on the severity of hyperbilirubinemia. J Clin Neonatol. 2020;9(1):46-51.

2.Babaei H, Parham S, Mohammadi Pirkashani L. Risk Factors of Severe Hyperbilirubinemia in Neonates Undergoing Exchange Transfusion in Imam Reza Hospital Kermanshah-Iran, during 2012 to 2016. Int J Pediatr. 2018;6(8):8061-72.

3.Boskabadi H, Rakhshanizadeh F, Zakerihamidi M. Evaluation of Maternal Risk Factors in Neonatal Hyperbilirubinemia. Arch Iran Med. 2020;23(2):128-40.

4.Mojtahedi SY, Izadi A, Seirafi G, Khedmat L, Tavakolizadeh R. Risk factors associated with neonatal jaundice: a cross-sectional study from Iran. Open Access Maced J Med Sci. 2018;6(8):1387-93.

5.Taksande A, Selvam S. Side Effects of Phototherapy in Neonatal Hyperbilirubinemia. Acta Sci Paediatr. 2018;1(5):24-30. Available from: https://www.actascientific.com/ASPE/pdf/ASPE-01-0039.pdf

6.Mohammadi Pirkashani L, Asghari G, Marofi M, Barekatain B. Effect of Chicory Extract Bath on Neonatal Bilirubin Levels: A Randomized Clinical Trial study. Int J Pediatr. 2017;5(12):6679-88.

7.Zahed Pasha Y, Ahmadpour-Kacho M, Ahmadi Jazi A, Gholinia H. Effect of Probiotics on Serum Bilirubin Level in Term Neonates with Jaundice; A Randomized Clinical Trial. Int J Pediatr. 2017;5(10):5953-8.

8.Ahmadpour-kacho M, Zahed Pasha Y, Ranjbar B, Pouramir M, Hajian K, Pounasrollah M. The Effect of Oral Zinc Sulfate on Serum Bilirubine Level in Term Neonates with Jaundice. Int J Pediatr. 2017;5(6):5053-60.

9.Kozgar SA, Chay P, Munns CF. Screening of vitamin D and calcium concentrations in neonates of mothers at high risk of vitamin D deficiency. BMC pediatr. 2020;20:332.

10.Sridhar NL, Sreeram S, Srinivas M. Is calcium a concern in neonates undergoing phototherapy?. Int J Contemp Pediatr. 2018;5(3):988-991.

11.Asl AS, Zarkeshl M, Heidarzadeh A, Maleknejad S, Hagikhani K. The effect of phototherapy on urinary calcium excretion in term neonates. Saudi J Kidney Dis Transpl. 2016;27(3):486-92.

12.Bhat JA, Sheikh SA, Wani ZA, Ara R. Prevalence of hypocalcemia, its correlation with duration of phototherapy and persistence in healthy term newborns after intensive phototherapy: A prospective observational hospital-based observational study. Imam J Applied Sci. 2019;4(2):57-61.

13.Maisels MJ, McDonagh AF. Phototherapy for neonatal jaundice. N Eng J Med. 2008;358:920-8.

14.Van Der Veere CN, Schoemaker B, Bakker C, Van Der Meer R, Jansen PL, Elferink RP. Influence of dietary calcium phosphate on the disposition of bilirubin in rats with unconjugated hyperbilirubinemia. Hepatology. 1996;24(3):620-6.

15.Singh A, Jialal I. Unconjugated Hyperbilirubinemia. Treasure Island (FL): StatPearls; 2020.

16.Dickson FJ, Sayer JA. Nephrocalcinosis: A Review of Monogenic Causes and Insights They Provide into This Heterogeneous Condition. Int J Mol Sci. 2020;21(1):369.

17.Olusanya BO, Osibanjo FB, Slusher TM. Risk factors for severe neonatal hyperbilirubinemia in low and middle-income countries: a systematic review and meta-analysis. PLoS One. 2015;10(2):e0117229.

18.Ip S, Chung M, Kulig J, O'Brien R, Sege R, Glicken S, et al. An evidence-based review of important issues concerning neonatal hyperbilirubinemia. Pediatrics. 2004;114(1):e130-53.

19.Vuralli D. Clinical approach to hypocalcemia in newborn period and infancy: who should be treated?. Int J Pediatr. 2019;2019:4318075.

20.Cuperus FJ, Iemhoff AA, Verkade HJ. Combined treatment strategies for unconjugated hyperbilirubinemia in gunn rats. Pediatr res. 2011;70(6):560-5.

21.Hafkamp AM, Havinga R, Sinaasappel M, Verkade HJ. Effective oral treatment of unconjugated hyperbilirubinemia in Gunn rats. Hepatology. 2005;41(3):526-34.

22.Van der Veere CN, Jansen PL, Sinaasappel M, Van der Meer R, Van der Sijs H, Rammeloo JA, et al. Oral calcium phosphate: a new therapy for Crigler-Najjar disease?. Gastroenterology. 1997;112(2):455-62.