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Original Article

Prophylactic effect of zinc sulphate on hyperbilirubinemia in premature very low birth weight neonates: a randomized clinical trial

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ABSTRACT

Background: One of the common problems in neonatal period is jaundice that occurs in the first week of birth in 60% of term and 80% of preterm neonates. In preterm newborn hyperbilirubinemia is higher, persistent, longer, and more likely to be associated with neurological injury than term neonates. The purpose of this study was to determine Prophylactic effect of zinc sulphate on hyperbilirubinemia in premature very low birth weight neonates.

Methods: Sixty Newborns who admitted in our NICU which had inclusion criteria were eligible in this trial. Included neonates were randomly placed in two groups (case and control) and before intervention the total serum bilirubin (TSB) was measured at second day. The participant received either 20 mg of zinc sulfate or placebo through NG-tube divided in two doses till day seven of age. Then total and indirect bilirubin was measured at 3ed, 5th and 7th day of life. If any of newborns in duration of hospitalization develop clinical jaundice, after assessment of bilirubin, need for phototherapy was evaluated based on phototherapy and exchange schedule as described by the American Academy of Pediatrics guidelines. The termination point of phototherapy was defined as a bilirubin level less than 50 percent of starting point. After gathering Data, they were analyzed using SPSS software (version 11.5) and T-test, Chi-square and repeated measurement tests.

Results: Seventy eight patients enrolled in this trial that 18 cases were excluded and the remaining cases divided into two equal groups (N=30 in each group). Demographic condition was similar in two groups. There were no different between two groups in decreasing total serum bilirubin and duration of phototherapy.

Conclusion: This study showed that zinc sulfate has no preventing effective in hyperbilirubinemia in preterm very low birth weight neonates. It has also no effect on duration of phototherapy.

Keywords: Jaundice, Phototherapy, Premature, Zinc

Introduction

One of the common problems of neonatal period is increased serum bilirubin level and clinical jaundice that occurs in the first week of birth in 60% of term and 80% of preterm neonates and usually benign (1). In preterm neonates, hyperbilirubinemia is higher, persist ant, longer and more likely to be associated with neurological injury than term neonates (2-4). The increased intensity and duration of hyperbilirubinemia in preterm infants as well as immaturity of the blood – brain barrier have led to concern about greater risk of bilirubin encephalopathy in preterm infants (1, 5). The treatment of hyperbilirubinemia is so necessary which is composed of three main procedures: mechanical removal of bilirubin, photo isomerization and bilirubin excretion in stool or urine and accelerating normal metabolic pathway for bilirubin excretion pharmacologically. Exchange transfusion is an invasive procedure with many complications and one percent mortality. The most common approach for treatment and prevention of neonatal hyperbilirubinemia is phototherapy, which reduces the incidence of exchange transfusion. This method has some disadvantages including parental anxiety due to increased hospitalization of the infant, disrupted mother-infant bonding and high cost of care (6, 7).

Deficient uridin glucuronyl transferase (UDPG-T) activity that results bilirubin conjugation impairment has long been considered a major cause of physiologic jaundice. In the first 10 days

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of life the UDPG-T activity in full term and premature neonates is usually less than 1% of adult values (3, 8). Numerous pharmacologic agents are capable to stimulate the hepatic glucoronid conjugating system and some has been used in attempts to reduce serum bilirubin concentration in newborn infants (6, 7, 9-12).

Inhibition of enterohepatic circulation is one of the therapies being tried for prevention of neonatal hyperbilirubinemia. Various substances have been used to bind the bilirubin in intestinal lumen to resist its absorption and prevent enterohepatic circulation. Seen as effective therapy, products such as oral agar have been used, but, with inconsistent results. Zinc has also been investigated for its role in decreasing the STB levels by inhibiting enterohepatic circulation (14, 15).

The aim of this study was to determine the prophylactic effect of zinc sulfate on hyper-bilirubinemia in very low birth weight (VLBW) neonates.

Material and Method

This randomized double blind clinical trial was performed on 60 very low birth weight neonates who admitted at our hospital.

This investigation was approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran. The procedure was explained completely to the guardians and written informed consents were obtained before their participation.

Healthy breastfed neonates with birth weight equal to or less than 1500 grams were selected from patients admitted in NICU since May 2013 to January 2014. Babies with congenital anomalies, intolerance feeding, GI anomalies, ventilator support, hemolytic disease and infections were excluded. Laboratory investigations included complete blood count, red blood cell morphology, blood groups of the newborn and their mothers, direct and indirect combs' tests, reticulocyte count and erythrocyte G6PD level. The clinical examination, gestational age, birth weight, sex, serial total serum bilirubin (TSB), direct bilirubin, duration of phototherapy and hospi-talization were recorded. TSB was measured by using a Unistatbilirubineometer (Reichert – Jung, Germany). Evaluation of inclusion criteria and performing the procedures was accomplished by a resident of neonatology who was calibrated by two academic staffs. The colorimetric method of Lathe and Ruthven were used for measurement of direct bilirubin (16).

Zinc sulfate and placebo were prepared in bottles with same shape, same volume and same taste. Since they were characterized with different codes as A and B. so for randomized allocation they were prescribed as decussates to patients. Both physicians and laboratory staff did not know about type of administration for each neonate. Codes were opened at the end of study.

As all neonates were healthy preterm babies, phototherapy was started when total serum bilirubin concentration reached a threshold level based on AAP recommendation (TSB 5 grams/dl in babies with less than 750 grams birth weight, TSB 6 grams/dl in 751-1000, TSB 7 grams/dl in 1001-1250 and TSB 8 grams/dl in more than 1250 grams birth weight) (4). Phototherapy was discontinued when bilirubin decreased to 50% of starting level. Each phototherapy unit contained six blue lamps. Energy output or irradiance of the phototherapy light was being maintained at 8-12 µw/Cm2/nm. Level irradiance of phototherapy was checked routinely by fluoro-LITE meter 451, Minolta camera co. LTD, and maintained at 8 - 12 µW/Cm2/nm.

To facilitate the selection of patients for exchanging based on existing standards, the following categorization was used by the authors: (TSB 13 grams/dl in babies with less than 750 grams birth weight, TSB 14 grams/dl in 751-1000, TSB 15 grams/dl in 1001-1250 and TSB 16 grams/dl in more than 1250 grams birth weight) (13).

All neonates were initially breastfeeding as minimal feeding with oro-gasteric tube, and then TSB levels were measured on second day. If TSB level was less than the phototherapy threshold values, patients were randomized in two groups.

Each patient received solutions as a twice doses (20mg zinc sulfate daily) by orogastric tube at second day of age. Solutions were prescribed until seventh day and after that were discontinued. Total serum bilirubin level (TSB) was checked before intervention, at onset of phototherapy, and 3rd, 5th, 7th day of intervention and end of ohototherapy.

Duration of phototherapy was calculated from onset to end of phototherapy or up to seventh day of trial. And the time of the beginning of phototherapy was calculated from birth time.

The results were analyzed IBM SPSS version 16 software (SPSS Inc, Chicago, IL). Quantitative variables are presented as Mean± Standard deviation. The normal distribution of variables was proved by Kolmogorov-Smirnov test. We used "Independent Samples T test" and Mann-Whitney for comparison of the variables with and without normal distribution, respectively. Pearson and Chi-square test were used for comparison of qualitative variables. Repeated measure ANOVA was used with a significance set level at $P<0.05$.

Results

In this randomized clinical trial, 78 patients enrolled which 18 cases of them was excluded duration the study because of evidence of gastrointestinal intolerance, parents refusing further treatment, infant death, symptoms of sepsis and lack of access in duration of treatment. Then, 60 remaining cases were divided into two groups (case group and control group). Demographic factors of mothers and newborns were the same in both groups. Also the condition of delivery, intrauterine growth and diseases in pregnancy period were similar in all patients. There were no significant differences between two groups regarding sex (case group: M: 33.3% F: 66.7% control group: M: 46.7% F: 5303%) and delivery method (both group were same: CS: 93.3% NVD: 6.7%). Mean gestational age were 31.8±2.22 and 30.4±2.04 weeks (P-value 0.014) and Mean birth weight were 131707±169.09 and 1260.33±170.83 grams (P-value 0.197) in case group and control group respectively.

After completion of project, by using t. test revealed no significant differences between two groups in these variables: at beginning time of phototherapy (P-value=0.228), duration of phototherapy (P-value=0.628). Other variables including: bilirubin level of third, fifth, seventh days and end of phototherapy have been explained at Table 1. As well as repeated measure analysis showed the absence of significant difference between treatment methods in two groups.

Figure 1 show that the total serum bilirubin levels of case group and control group on all days of the study, although TSB was different in two Group but by using repeated measure analysis ANOVA was not statistically differences.

Table 1. Changes in total serum bilirubin (TSB) levels after intervention

Plasma bilirubin level (mg/dl)	Case group (n=30) Mean (SD) Range	Control group (n=30) Mean (SD) Range	P value
TSB before intervention	6.89 (0.89) 4.8-8.9	6.19 (1.37) 3.9-8.9	0.022
TSB at onset of phototherapy	8.39 (1.24) 5.7-11.5	8.09 (1.29) 6.1-12	0.354
TSB at third day	9.41 (2.27) 5.9-14.7	8.96 (2.56) 4.3-14.5	0.481
TSB at fifth day	7.2 (2.6) 1.5-12.8	5.8 (1.9) 2.7-9.6	0.022
TSB at seventh day	5.98 (2.47) 1.6-13.1	5.71 (2.24) 1.9-11.5	0.660
TSB at end of phototherapy	3.27(0.94) 1.5-4	3.1 (0.23) 2.8-3.5	0.676

Discussion

The effects of numerous drugs on bilirubin metabolism has been identified such as meta-lloporphyrins and depenicilamin by inhibition of hemeoxygenase, agar and charcoal by decreasing entrohepatic circulation (3).

Zinc salts have been demonstrated to be promising in both *in vitro* and *in vivo*. In other words, the action of zinc in hyperbilirubinemia depends on its ability to reach the distal intestine where it is once again absorbed into the blood and therefore reduces the enterohepatic circulation (17).

There are limited studies regarding the efficacy of zinc in Reducing Hyperbilirubinemia among preterm neonates, however until now, it isn't available any report in VLBW neonates.

Current research revealed that administration of 20mg oral zinc (zinc sulfate) as twice doses in VLBW neonates did not reduce the incidence of subsequent hyperbilirubinemia during first week of age.

Rana et al concluded that twice daily administration of oral zinc in a dose of 10 mg dose not reduce the incidence of hyperbilirubinemia in in term and late preterm (>35 weeks) between 25 and 168 h of age. They also showed that zinc administration was not associated with any reduction in proportion of neonates requiring phototherapy; however, duration of phototherapy was lower in oral zinc supplemented group compared to placebo group (17).

Similarly, in present study administration of oral zinc have not effect on prevention of hyper-bilirubinemia, unlikely, duration of phototherapy was same between two groups.

Patton et al in a study on term neonates showed that oral administration of zinc in a dose of 5mg/twice daily did not create significant difference in duration of hyperbilirubinemia, in an

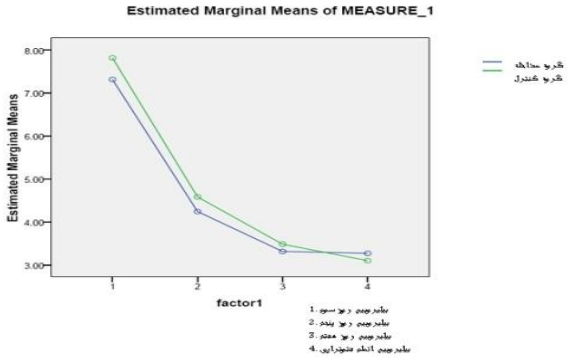

Figure 1. Estimated Marginal Means of Two Groups

another investigation on term neonates (18), Mamoori et al concluded that zinc administration did not reduce the severity of jaundice and also did not delay onset of it, but need for phototherapy and the hours of phototherapy in the group receiving the zinc was clearly lower than control group (19).

The basis for why authors administrated zinc in this dose comes from several trials treating large number of children with diarrhea, measles, pneumonia, common cold and malaria in children that has shown oral zinc in this dose to be quite safe (20–22). The role of zinc in hyper-bilirubinemia depends on its ability to reach the terminal ileum where it precipitates the unconjugated bilirubin to prevent the enterohepatic circulation, but, ineffectiveness in reduction of incidence of hyperbilirubinemia and need for phototherapy in this study could be delayed administration time (second day).

A higher bilirubin level before intervention and grater gestational age in case group compared with control group, Maybe another reason for lack of efficacy of zinc sulfate in reducing the amount of bilirubin. Certainly, more similarity in these variables in two groups will be helpful for accurate assessment.

Another possibility could be absorption of oral zinc salt in proximal intestine leading to its unavailability in the distal intestine, where it should actually reach to prevent enterohepatic circulation of unconjugated bilirubin. There could be a role of zinc preparation which is either non-absorbable or slowly absorbable. There could also be a scope of higher dose or more frequent administration of oral zinc or another zinc salts. However, all these speculations require further exploration.

Bilirubin level at onset of phototherapy in preterm neonates is lower than term infants so because of this reason the zinc element doses not have enough time for performing the prophylactic effect on hyperbilirubinemia. On the other hand as the preterm newborn is zinc deficient second to preterm delivery it appears that zinc before reaching to intestine for bonding to bilirubin has been absorbed. We followed our babies for only 7 days. We think if we did more days treatment may be to get prophylactic effect.

The authors do not know the pre- and post-supplementation levels of zinc in neonates. Monitoring serum zinc in enrolled neonates could be informative in relation to the details of complex formation between zinc and bilirubin; however, it could not be done due to logistic reasons. The data regarding the biochemical interactions of zinc and bilirubin are scarce. Indirect evidences are available from the in vitro studies of Mendez-Sanchez et al (15) and Vitek et al (23).

Another possibility of the results of this study is small sample size. There were no significant adverse effects in the zinc group. The incidence of vomiting, diarrhea, rash, seizures, and sepsis were not higher in the zinc group compared to the placebo group.

There was no mortality in either group throughout the study period. In all, zinc appeared to be a safe drug. There are few limitations in the current study. Firstly, dose of oral zinc was arbitrarily decided as 10 mg/day based on previous studies in older children; however, results could have been different with use of different dose of oral zinc. Secondly, a pilot trial comparing different formulations would have helped in deciding the better formulation of oral zinc. Finally, starting the therapy earlier e.g. before 12–24 h of age may be better for prevention of hyperbilirubinemia. To summarize, administration of oral zinc salt in a dose of 20 mg/day, divided in two doses from second to seventh days of age did not reduce the duration of phototherapy; and, it did not decrease the incidence of hyperbilirubinemia during the first week of life.

Conclusion

According to premature and VLBW newborns in this study, and early onset of jaundice which requiring phototherapy in these patients, so administration of zinc hadn't obvious prophylactic effect on creating jaundice or reducing TSB level during treatment. Due to mechanism of action of zinc on reducing enterohepatic circulation, it seems to obtain positive results in VLBW neonate longer treatment, increased doses, using another zinc salts and increased sample size may be necessary.

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