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Original Article

Effect of oral zinc on hyperbilirubinemia in full term neonates

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Abstract

Background Oral zinc has been shown to reduce serum unconjugated bilirubin in animals, adolescents and low birth weight neonates. However, studies in healthy term neonates given oral zinc showed no reduction in hyperbilirubinemia based on time measurement in days. In order to improve accuracy, hyperbilirubinemia may be determined based on time measurements in hours.

Objective To determine the effect of oral zinc on hyperbilirubinemia in full term neonates, based on time measurement in hours, rather than days.

Methods We conducted a randomized, double-blind clinical trial on healthy term neonates born spontaneously or through elective caesarean section in Hasan Sadikin Hospital from June to July 2010. Subjects were randomized into two groups: those receiving 5 mg of zinc sulphate and those receiving a placebo, sucrose, each twice daily. Serum total bilirubin level was examined at discharge and upon follow-up at day 5 of life. Factors which may be related to hyperbilirubinemia such as maternal age, infants' gender, umbilical cord bilirubin levels and type of feeding, were analyzed by chi-square test. Hyperbilirubinemia persistence and comparison of survival distributions were analyzed by Kaplan-Meier survival analysis and Logrank test.

Results Out of 60 subjects, 26 had hyperbilirubinemia. The mean duration of hyperbilirubinemia in the 15 subjects in the zinc group and 11 in the placebo group were 116.5 hours and 117.3 hours, respectively. There was no significant difference in hyperbilirubinemia duration between the two groups ($P=0.496$, 95% CI 111.5 to 122.7). In addition, chi-square analysis of factors which may be related to hyperbilirubinemia showed no significant difference between the two groups ($P > 0.05$).

Conclusions Oral zinc 5 mg twice daily made no significant difference in hyperbilirubinemia duration in full term neonates despite measuring in hours. **[Paediatr Indones. 2011;51:107-10]**.

Keywords: *full term neonates, hyperbilirubinemia, oral zinc, survival analysis*

Bilirubin enterohepatic circulation is one determinant of neonatal hyperbilirubinemia.[1-3] Studies on animals, adolescents and low birth weight neonates showed that oral zinc salt intake decreased serum bilirubin levels, perhaps through inhibition of the bilirubin enterohepatic circulation.[4-6] In contrast, a study on at risk healthy term neonates showed that oral zinc did not reduce hyperbilirubinemia in the first week of life.[7] Since these results were inconsistent, we conducted this study to determine the effect of oral zinc on hyperbilirubinemia in full term neonates using time duration measured in hours.

Methods

This randomized, double-blind clinical trial on healthy term neonates who were born spontaneously or through elective caesarean section was conducted at the perinatology ward of Hasan Sadikin Hospital, Bandung from June to July 2010. We included term appropriate for gestational age neonates from singleton

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pregnancies, with umbilical cord total bilirubin $\geq$ 2 mg/dL. We excluded those whose mothers had negative Rhesus factor, O blood type or complicated pregnancies, and babies with congenital anomalies. This study was approved by Ethics Committee of Hasan Sadikin Hospital/ Medical School, Padjadjaran University .

Due to the difficulty in determining hyperbilirubinemia mean survival time, we used two proportion formula to determine sample size instead of survival analysis. The required sample size was 30 subjects for each group to get 95% confidence interval and 80% power test. Subjects were collected by consecutive sampling and randomized with block permutation.

We interviewed mothers to obtain information on maternal age, past illnesses, pregnancy history, drug consumption, history of icterus in previous children, blood type and Rhesus factor. Written informed consent was obtained from parents. After delivery, we recorded APGAR scores and birth weights, performed physical examinations, checked umbilical cord blood total bilirubin levels and determined infant gestational ages by computing from the first day of the last menstrual period and New Ballard score.

Subjects were registered and divided into two groups: the intervention group receiving zinc syrup and the placebo group receiving sucrose syrup, each given twice daily for five days. The syrup supplements were prepared by Hasan Sadikin Hospital Pharmacy Department, 1.25 mL zinc syrup (coded A) containing 5 mg zinc sulphate and 1.25 mL placebo syrup (coded B) containing sucrose. The placebo was identic in color, appearance and packaging to the zinc sulphate. Syrup supplements were given soon after the first feeding and continued for 5 days by the nurse for subjects in the perinatology ward, or by the mother after discharge from the hospital. Subjects' serum total bilirubin were checked at the time of discharge and day 5 of life. At day 5, we measured subjects' body weights, performed physical examinations, interviewed mothers about type of feeding, checked their compliance in giving the supplements by measuring the remaining syrup volumes, and obtained subjects' history of vomitus or diarrhea at home as possible adverse effects of zinc. We also visited subjects at home for those unable to return for the day 5 check. The primary outcome measure was deemed to be the incidence of hyperbilirubinemia, defined as total serum bilirubin $\geq$ 13 mg/dL anytime between days 1 and 5 of life. Secondary outcome measures were the mean duration of hyperbilirubinemia and the proportion of subjects requiring phototherapy.

Factors related to hyperbilirubinemia (maternal age, gender, umbilical cord bilirubin and type of feeding) were analyzed with Pearson's chi-square test. Hyperbilirubinemia persistence based on time in hours was analyzed with Kaplan-Meier survival analysis. We compared both groups' survival distribution with Log-rank test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS version 13.0 for Windows, SPSS Inc, Chicago-Illinois, USA.

Results

We divided the subjects into two groups of 30 each, one group receiving zinc and the other receiving sucrose as a placebo. General characteristics of subjects are listed in **Table 1**.

Of the 60 subjects, 26 had hyperbilirubinemia: 15 in the zinc group and 11 in the placebo group. Factors related to hyperbilirubinemia were analyzed using chi-square test shown in **Table 2**.

Table 1. Characteristics of subjects.

	Group	
	Zinc (n = 30)	Placebo (n = 30)
Mean gestational age, months (SD)	39.1 (1.1)	39.2 (1.3)
Mean birth weight, kg (SD)	3 (0.3)	3.1 (0.4)
Delivery type		
Spontaneous	24	17
Caesarean section	6	13

Note: SD = standard deviation

Table 2. Characteristics of the 26 subjects with hyperbilirubinemia.

	Hyperbilirubinemia		P*
	Zinc (n = 15)	Placebo (n = 11)	
Maternal age (year)			
< 25	7	2	0.195
≥ 25	8	9	
Gender			
Male	7	6	0.746
Female	8	5	
Umbilical cord bilirubin , mg/dL			
2 - < 2.5	11	5	0.425
≥ 2.5	4	6	
Type of feeding			
Breast milk	9	2	0.127
Breast milk and formula	7	9	

Note: *chi-square test

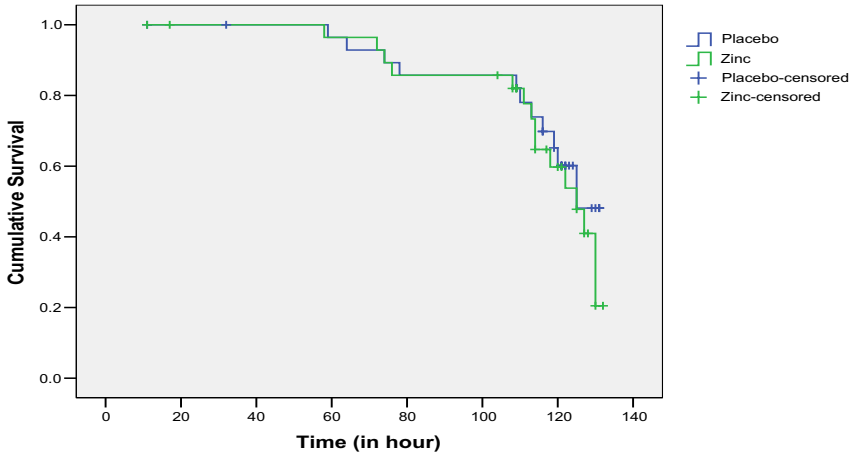

Figure 1. Hyperbilirubinemia survival analysis in zinc and placebo groups

By Kaplan-Meier survival analysis, we found hyperbilirubinemia first occurred at 58 hours and last occurred at 130 hours, with a mean time of 116.5 hours in the zinc group. In the placebo group, hyperbilirubinemia first occurred at 59 hours and last occurred at 125 hours, with a mean time of 117.3 hours. Survival analysis plot is shown in **Figure 1**. Test of equality of survival distributions by Logrank test showed no significant difference in the groups (P = 0.496).

Discussion

Bilirubin is produced by the catabolism of heme in the reticuloendothelial system. This bilirubin is in an unconjugated form and is released into the circulation and transported to hepatocytes where it combines enzymatically with glucuronic acid, producing bilirubin mono- and diglucuronides. The conjugation reaction is catalyzed by uridine diphosphate glucuronosyltransferase (UGT-1A1). Mono- and diglucuronides are excreted into the bile and the gut. In newborns, much of the conjugated bilirubin in the intestine is hydrolyzed back into unconjugated bilirubin (UCB), a reaction catalyzed by the enzyme beta-glucuronidase present in intestinal mucosa. UCB is reabsorbed into the blood stream by way of the enterohepatic circulation.[1,2] Previous studies have proposed that oral zinc will bind UCB

in the intestine to form a structure that cannot be reabsorbed by intestine into the blood stream, such that the structure will be excreted in the faeces causing UCB blood levels to decrease.[4,5] Based on this theory, we expected oral zinc to reduce hyperbilirubinemia. However, this was not the case, as no significant difference was found in hyperbilirubinemia duration between groups (P = 0.496). Our discrepant results might be caused by several factors affecting neonatal hyperbilirubinemia, e.g. maternal age, subject gender, umbilical cord total bilirubin level, and type of feeding. However, we found no significant difference in the two groups for any of these factors.

Pharmacokinetic and pharmacodynamic factors may also affect a subject's response to zinc.[8] We assumed there were no pharmacokinetic factors affecting our results, as all subjects had similar physiologic conditions, all parents gave the syrup supplement regularly and in equal dosage, no adverse effects were reported in the zinc group and all subjects were in good health throughout the study. Likewise, no subjects took other medications, thus ruling out drug interactions affecting the results. However, pharmacodynamics may have affected our study. Mendez-Sanchez *et al*.[5] gave single dose oral zinc sulphate at 40 mg and 100 mg daily for 7 days to adult Gilbert's syndrome patients. They showed a significant decrease in bilirubin serum levels in their subjects. In our study, we gave 5 mg zinc sulphate orally twice daily for five days with no significant decrease in hyperbilirubinemia. It is possible that study results were skewed by low dosage of the zinc sulphate and/or the small sample size of our study.

In contrast to previous studies[5-7] which observed the effect of oral zinc in reducing UCB serum levels based on time measured in days, we measured time in hours. In Rana's study[7] in India, oral zinc gluconate was given to subjects starting on the second day of life, whereas, we gave oral zinc soon after the first feeding and observed hyperbilirubinemia based on time in hours and analyzed hyperbilirubinemia duration with survival analysis. We found 2 and 4 subjects in the zinc and in placebo groups, respectively, that required phototherapy. In agreement with Rana's study, the need for phototherapy not significantly different between the two groups.

There were limitations in our study. For example, we did not check the zinc serum levels before and after intervention. Nor did we check zinc and bilirubin levels in faeces in our subjects due to limited laboratory facilities. We conclude there was no difference in hyperbilirubinemia duration in full term neonates who received oral zinc versus those who received a placebo.

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