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Original Article

The role of zinc supplementation in humoral immune response to hepatitis B vaccination in infants: a double-blind randomized placebo-controlled trial

K. Suarca, Hendra S, B. N. P. Arhana, I K. G. Suandi

Abstract

Background Suboptimal zinc intake may depress thymus function, lymphoproliferation, and T cell-dependent B-cell proliferation, which can impair antibody production. Zinc supplementation can improve immune function and reduce morbidity.

Objective To assess the effect of zinc supplementation on infants' anti-HBs titer after hepatitis B vaccination.

Methods A double-blind randomized control trial of 66 healthy infants in Pustu Dauh Puri, Denpasar Barat was conducted. Subjects were followed from birth to three months of age and were placed into two treatment groups using block randomization. One group received zinc supplements with a standard hepatitis B vaccination (zinc group, n=33) and the other group received placebo supplements with standard hepatitis B vaccination (placebo group, n=33). The serum zinc levels were measured at baseline and at three months. The difference in levels of anti-HBs titer between the zinc and placebo groups was the primary endpoint of this study.

Results The serum zinc levels were significantly higher in the zinc group compared to the placebo group (P = 0.017), with a mean difference of 18.76 mIU/ml (95% CI 3.45 to 34.07). Regardless of baseline serum zinc levels, the mean anti-HBs titers were significantly higher in the zinc group compared to the placebo group (P <0.0001; mean difference = 495.8 mIU/mL; 95% CI 362.32 to 629.44). Multivariate analysis showed that zinc supplementation was the only variable that influenced anti-HBs titer levels (P < 0.0001).

Conclusion Regardless of their initial zinc serum level, infants receiving zinc supplements along with standard hepatitis B vaccination have significantly higher levels of anti-HBs titers than infants receiving hepatitis B vaccination without zinc supplements. **[Paediatr Indones. 2008;48:374-80]**.

Keywords: *zinc supplementation, serum zinc level, hepatitis B vaccination, anti-HBs titer*

Since the 1930's, zinc's importance as an essential human micronutrient has been well known. In developing countries, zinc deficiency has been linked to growth delay and increased morbidity and mortality from infectious disease due to impaired immune system development in infants and children.[1,2] About 82% of lactating mothers world-wide are likely to have inadequate zinc intake.[3] In Indonesia, specifically, researchers found that zinc deficiency occur in 25% of lactating mothers and 17% of their infants.[4] Dorea (2002) found that clinical symptoms associated with zinc deficiency often occurred in breastfed infants, and compared to infants born at term, symptoms occurred earlier in preterm infants.[5]

Insufficient dietary zinc intake can lead to impaired immune function due to depressed thymus activity, causing decreased lymphoproliferation and T lymphocyte development, which also has the affect of decreasing antibodies due to impaired T dependent

From the Department of Child Health, Medical School, Udayana University, Sanglah Hospital, Denpasar, Indonesia.

Request reprint to: Kadek Suarca, MD, Department of Child Health, Medical School, Udayana University, Sanglah Hospital, Jl. Pulau Nias Denpasar Bali, Indonesia. Tel. 62-361-244038. Fax. 62-361-244038. E-mail: kiul1912@yahoo.com

B-cell activation.[6] Zinc supplementation is not only a treatment for malnourished or zinc deficient children. In children with normal serum zinc levels, but who are susceptible to zinc deficiency (e.g. infants aged 1 to 4 months with inadequate zinc intake), zinc supplementation can also be beneficial.[1,2,4,7]

Vaccination is used to induce the production of lymphocytes, antibodies, and memory cells that are sensitive to a specific antigen. This process can be disturbed if zinc intake is suboptimal or deficient.[8] Thus, efforts must be made to ensure that at the time of vaccination, patients' zinc levels should be adequate to ensure maximal antibody production and possibly, longer immune protection.

To assess the immune response to vaccination, a measurement for antibody titer is needed. Recently, a technique involving Depnasar was developed and can be used to measure hepatitis B antibody (anti-HBs) titers. The aim of this study is to investigate the effect of oral zinc supplementation on antibody titer after standard hepatitis B (HB) vaccinations in healthy infants.

Methods

Design and subjects

This was a double-blind randomized placebo-controlled trial conducted between February 2005 and May 2006. Eligible subjects were recruited consecutively from all healthy babies born at Puskesmas Pembantu Dauh Puri Denpasar Barat who met the inclusion criteria. The study received ethical clearance from the Ethics Commitee of Udayana Medical School/Sanglah Hospital Denpasar.

We included all babies who were born from spontaneous delivery, at term and were vigorous, with a birth weight between 2500 and 4500 gram. The parents of all subjects also gave informed consent. Infants with a major congenital defect or a mother with acquired immune deficiency syndrome (AIDS), history of jaundice or positive HBs-Ag titer, history of immunosuppressive therapy during pregnancy, or a history of blood transfusion in the last three months were excluded. The subjects were assigned by means of block randomization to either receive zinc or placebo.

Definition of variables

Zinc status was determined based on serum zinc level. Zinc deficiency was defined as a serum zinc level <49.7 μg/dl in neonates and <64.5 μg/dl in infants and preschool-aged children.[9] The immune response to HB vaccination was determined by measurement of the anti-HBs titer a month after HB vaccination was completed. HB vaccination was given according to a standard vaccination protocol for public health centers. The length of an episode of diarrhea was measured from the first occurrence of diarrhea until recovery. The frequency of episodes of diarrhea was counted as the number of diarrhea episodes experienced during the study. Subjects were deemed compliant if the medicines were not taken less than or equal to seven days on successive days in one month, or less than or equal to 10 days on nonsuccesive days in one month. Subjects were deemed as noncompliant if the medicines were not taken more than seven days on succesive days in one month, or more than 10 days on nonsuccessive days in one month. The type of oral nutrition the infant received during the study period was classified as either exclusive breastfeeding or nonexclusive breastfeeding.

Data collection

Baseline characteristics, clinical data, time of HB vaccination, nutrition type (breastfed vs. not breastfed), episodes of diarrhea, body weight, follow-up, laboratory results, and side effects were collected from subjects' medical charts and were recorded using a standardized questionnaire.

Sample size

Sample size was calculated to obtain a power of 90% at a significance level of 5% ($P<0.05$). To obtain this power and level of significance, we would need 33 subjects per group. We intended to identify effects of 100 mIU/ml difference in anti-HBs response between the zinc and placebo groups. Due to the absence of previous data, to determine the pooled standard deviation, a pilot investigation was performed on 20 samples and a pooled standard deviation for the anti HBs titer of 129 mIU/ml was obtained.

Randomization

Subjects were allocated using a block randomization method (six patients per block). Assignments were placed in closed envelopes, kept at the Pharmaceutical Installation of Sanglah Hospital Denpasar, and opened after the study ended.

Interventions

Oral zinc supplementation or placebo was administered soon after subjects were allowed oral intake. The zinc group received 60 mg zinc sulfate as a powder, given once a day for three months, and the placebo group received placebo (saccharum lactis) powder once a day for three months. The packaging, color, and aroma of the zinc sulfate and placebo powders were identical. The raw materials were purchased from a pharmaceutical distributor in Jakarta, and the materials were packaged by the Pharmaceutical Installation of Sanglah Hospital Denpasar. The packaging of zinc and placebo were coded A or B with a key, which was enclosed in an envelope and opened after the study was completed. The officer in charge, researcher, patient and parent did not know about the contents of each package. If the subject vomited within 30 minutes of drug administration, the medicine was re-administered.

Laboratory measurement

An initial blood specimen for baseline serum zinc level measurement was collected in Pustu Dauh Puri. At the end of study, a blood specimen used to test serum zinc levels and anti-HBs titers was collected during a home visit. Measurements of serum zinc levels and anti-HBs titers were performed in the Prodia laboratory. Serum zinc measurements were done using a colorimetric method with Zn 23411 Randox as a reagent. Anti-HBs titer were measured using the Micro particle Enzyme Immunoassay (MEIA) method with reagents from Ausab Axym Kit.

Follow-up

Information on diarrhea occurrence, nutrition type, body weight, HB vaccination schedule, compliance, and side effects was recorded in participants' medical charts by a health worker during weekly home visitation. We measured serum zinc levels and anti-HBs titers after 90 days of intervention. Subjects were excluded from the study if subject had a severe illness requiring hospitalization, suffered from jaundice or HB infection (detected by laboratory results), could not continue to take supplements or give blood samples, could not be vaccinated as scheduled, if their parents refused to continue the study, or if the home address could not be found during visitation.

Statistical analysis

Statistical analysis was performed using [insert same, version, and publisher of the statistical program used]. The significance level was set at $P<0.05$. Normality data were tested using a nonparametric Kolmogorov-Smirnov test. Independent sample t-tests were used to compare differences in zinc serum levels, anti-HBs titers, birth weights, present weights, and diarrhea episodes between the two groups. The effects of gender, zinc status, nutrition variety, and compliance were analyzed using a Chi-square test. Finally, we performed a multivariate analysis (ANCOVA) to determine if there were any confounding factors that may have influenced anti-HBs response.

Results

During the study period, 342 babies were born. There were 71 babies whose parents agreed to participate in this study, and 5 babies were excluded. Of the remaining 66 babies, 33 were placed in the zinc group and 33 in the placebo group (**Figure 1**).

The subject characteristics of infants in the zinc and placebo groups are shown in **Table 1**. The increase in body weight was significantly higher in the zinc group compared to the placebo group - 3427.2 gram (SD 580.83) vs. to 2628.7 gram (SD 452.08), respectively; mean difference = 798.49 (95% CI 542.52 to 1054.74); $P<0.0001$. At the end of the study, 36 babies (54.5%) were still exclusively breastfed.

Initially, there were four subjects (two babies in the placebo group, two babies in the zinc group) with zinc deficiency. By the end of the study, eight babies (seven babies in the placebo group and one baby in the zinc group) exhibited zinc deficiency (data not

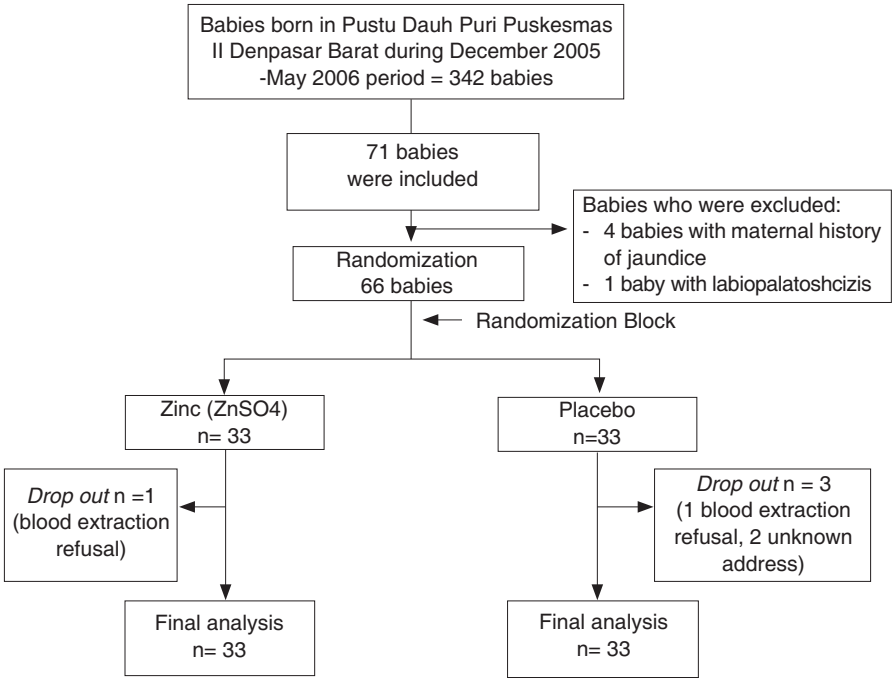

Figure 1. The study results

Table 1. Subject characteristics

Characteristics	Zinc Group (n=33)	Placebo Group (n=33)
Sex; male, n(%)	12 (36.4)	17 (51.5)
Birth weight (g); mean (SD)	3106.1	3131.8
Present weight (g); mean (SD)	(381.6)	(304.9)
Age at the time of vaccination	6533.3	5760.6
Hepatitis B1 (day), mean (SD)	(477.7)	(416.8)
Hepatitis B2 (day), mean (SD)	1.3 (0.54)	1.1 (0.77)
Hepatitis B3 (day), mean (SD)	30.0 (1.73)	30.2 (1.73)
Initial zinc level; normal, n(%)	60.4 (2.32)	60.6 (1.71)
The end zinc level; normal, n(%)	31 (93.9)	31 (93.9)
Anti-Hbs production response	32 (97.0)	26 (78.8)
High responder, n(%)	9 (27.3)	1(3.0)
Good responder, n(%)	23 (69.7)	9 (27.3)
Low responder, n(%)	0	14 (42.4)
Non responder, n(%)	1 (3.0)	9 (15.2)
Nutrition; exclusive breastfeeding, n(%)	21 (63.6)	15 (45.5)
Episode of diarrhea (time), mean (SD)	0.3 (0.8)	1.3 (2.0)
Compliance, n(%)	32 (97.0)	29 (87.9)

SD = Standard Deviation; n = sample size

shown). The serum zinc concentration increased in the zinc group from 79.7 (SD 19.97) μg/dl to 102.1 (SD 29.48) μg/dl, while in the placebo group, the concentration level went from 79.3 (SD 19.13) μg/dl at baseline to 82.9 (SD 23.46) μg/dl by the end of the study. When comparing increases in serum zinc levels, we found a significant difference between babies in the zinc group and the placebo group - 22.3 (SD 29.92) μg/dl vs. 3.6 (SD 32.29) μg/dl, respectively; mean difference = 18.7 μg/dl (95% CI 3.45 to 34.07); P=0.017 (**Figure 2**). Because there was an insufficient number of babies who exhibited zinc deficiency, a subgroup analysis was not conducted.

Regardless of initial serum zinc levels, by the end

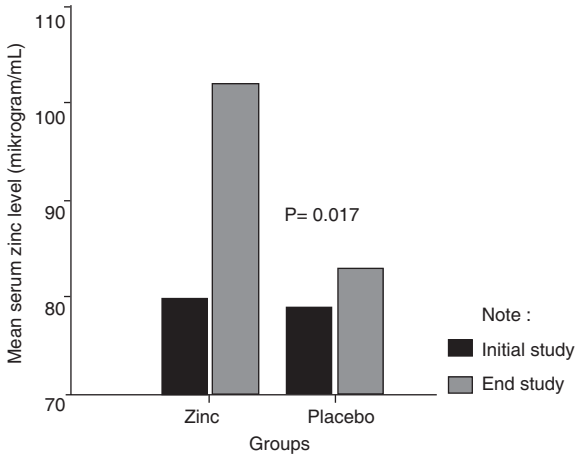

Figure 2. Mean serum zinc level in the two groups

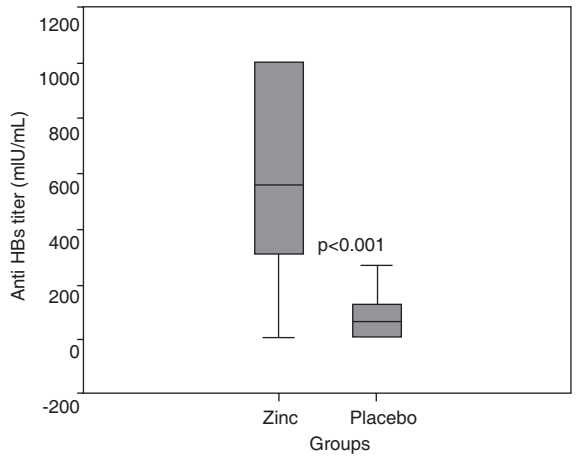

Figure 3. Anti-HBs titer profile after zinc supplementation

of the study, the mean anti-HBs titers were significantly higher in infants in the zinc group compared to those in the placebo group - 606.8 mIU/ml (SD 336.02) vs. 110.9 mIU/ml (SD 181.33), respectively; mean difference = 495.8 mIU/ml (CI 95%: 362.32-629.44); P<0.0001 (**Figure 3**).

The associations of several confounding factors that may have influenced anti-HBs titers are shown in **Table 2**. No side effects were reported in this study.

Table 2. The relationship between certain variable and anti HBs titer

Variable	F	P
Compliance	1.271	0.26
Nutrition	2.682	0.10
Diarrhea episode	0.047	0.83
Initial serum zinc level	1.582	0.21
Zinc supplementation	29.149	<0.0001

F = ANCOVA statistical value; P = probability

Discussion

Previous studies have reported that zinc supplementation increases nonspecific immune system and cellular immune responses.[7,11-13] To our knowledge, the role of zinc supplementation in the humoral immune response after vaccination in infants has not been reported. Several studies have been conducted looking at the role of zinc supplementation in the adult humoral immune response; albeit with controversial results. In a cohort of patients receiving repeated hemodialysis, Turk[14] found that there was no significant difference in the antibody response to a multivalent influenza vaccine between patients receiving zinc supplementation and those receiving placebo. In another cohort of hemodialysis patients, researchers found that zinc supplementation did not lead to an enhanced antibody response after HB revaccination.[15] Karlsen[7] found that zinc increased the intestinal antitoxin immune response in patients receiving oral cholera toxoid vaccination. A recent study from the United States concluded that zinc supplementation had no effect on antibody responses after pneumococcal conjugate vaccination in HIV positive adult patients.[16]

Using the 0, 1, and 2 months HB vaccination schedule, Kumar[17] found that 40 of 69 subjects had a 100% zero-protective titer at four weeks after the third dose, with a geometric mean concentration of 2643 mIU/ml. In our study, we found that after vaccination, anti-HBs mean titer was significantly higher in the zinc group than in the placebo group. We were not able to compare this result with other studies since there is no other report regarding the role of zinc supplementation in antibody response to vaccination in the first three months of life. This result strengthens the opinion that zinc supplementation can increase the humoral immune response after vaccination,[12] though there are several reports that zinc supplementation does not have a significant effect.[14,16,18] These prior reports have studied preschool and adult populations, however, thus, subjects in these studies have passed the highest growth acceleration period.[8] Zinc deficiency during this time may delay B lymphocyte responses due to impairment of B lymphocyte mitogen and plaque-forming responses.[13] In addition, passive immunity from maternal antibodies decreases in the first six months of life, suggesting that early effective intervention for zinc deficiency is needed to decrease morbidity in susceptible children.[2]

Bhandari[19] reported that zinc supplementation has beneficial effects as a preventive measure in children with normal serum levels. Zinc supplementation reduced mortality and morbidity rate due to various infectious diseases. Therefore, we suggest that food for babies and preschool children in developing countries be fortified with zinc.

At baseline, we found four infants with zinc deficiency in both the zinc and placebo groups. By the

end of this study, we observed one infant (zinc group) and seven infants (placebo group) with low serum zinc levels. Zinc supplementation led to significantly higher zinc levels in the zinc group compared to the placebo group (P=0.017). There were 54.5% of subjects who were still breastfed exclusively until the end of this study. A cross-sectional survey in West Java conducted by Dijkhuizen[4] found that 17% of babies and 25% of lactating mothers were zinc deficient. Lactating mothers with low serum zinc levels also have low zinc levels in their breast milk, which contributes to zinc deficiency in the infants.[20,21] Since the number of subjects with zinc deficiency were quite small in our study, we could not conduct a subgroup analysis to compare differences in outcomes between babies with normal and low serum zinc levels. Several studies have reported that zinc supplementation can increase growth and development in infants and children.[2,3,21-23] The results of our study corroborate the findings of earlier reports, as the increase in body weight was significantly higher in the zinc group compared to placebo group.

Side effects with zinc supplementation have not been reported in most studies.[2,7,23-26] No side effects were found in our study, either, though side effects of zinc supplementation may occur at very high doses.[9,27-29] The cause of side effects with zinc supplementation may be due to the zinc molecule's high affinity for electrons, which are present in several amino acids.[13]

Four subjects were excluded for reasons unrelated to zinc supplementation. Two babies were excluded because parents refused blood tests by the end of the study, and two babies were not included because their home addresses were unknown. Thus, intention-to-treat analysis was performed on the final data.

The effect of maternal zinc level on infant antibody response remains controversial. A study in Ecuador showed that there was no proof that zinc plays a role in antibody transfer from mother to her infant. Others studies suggest that zinc supplementation in pregnant women might produce a higher antibody titer in her baby.[1] Due to the limitation of resources we did not measure anti-HBs and HBs-Ag titer for babies and mothers at baseline. The antibody production response would be clearer if pre- and post-supplementation studies or repeated measurements were done. Due to the limitations of the antibody measurement tool, not all results could be presented with an absolute number. Therefore, we took a maximum and minimum value approach if the measurement value result was less than or exceeded the measurement tool capacity.

In conclusion, regardless of initial zinc serum levels, the antibody titer in response to HB vaccination in the zinc supplementation group was higher than in the placebo group. Furthermore, it was observed that zinc supplementation at the levels used had no side effects.

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