

Original Article

Maternal zinc status is associated with breast milk zinc concentration and zinc status in breastfed infants aged 4-6 months

Oraporn Dumrongwongsiri MD¹, Umaporn Suthutvoravut MD¹, Suthida Chatvutinun MS², Phanphen Phoonlabdacha MS¹, Areeporn Sangcakul MS³, Artitaya Siripinyanond PhD⁴, Usana Thiengmanee BSc⁴, Nalinee Chongviriyaphan MD, PhD¹

¹Division of Nutrition, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

²Pediatric Nursing Division, Nursing Service Department, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

³Research Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

⁴Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand

Breast milk provides adequate nutrients during the first 6 months of life. However, there are some reports of zinc deficiency in breastfed infants. This study was conducted to determine the prevalence of zinc deficiency in infants aged 4-6 months and the associated factors. Healthy infants aged 4-6 months and their mothers were enrolled. They were classified by feeding types as breastfed (BF), formula-fed (FF), and mixed groups (MF). Data collection included demographic data, perinatal data, given diets, and anthropometric measurement. Blood from infants and lactating mothers, and breast milk samples were collected to assess plasma and breast milk zinc concentrations. From 158 infants, the prevalence of zinc deficiency (plasma level below 10.7 $\mu\text{mol/L}$) was 7.6%, and according to feeding groups 14.9%, 5.3%, and 2.9% in the BF, the FF, and the MF groups, respectively. Breastfed infants with zinc deficiency had significantly lower maternal zinc concentrations compared with those without zinc deficiency. There was a higher proportion of maternal zinc deficiency in zinc-deficient infants than those without zinc deficiency (66.7% vs 16.2%, $p=0.02$). There was a positive correlation between zinc concentrations in breast milk and plasma zinc concentrations of infants ($r=0.62$, $p=0.01$) and plasma zinc concentrations of lactating mothers ($r=0.56$, $p=0.016$). Using the regression analysis, infant zinc status was associated with maternal plasma zinc concentrations among breastfed infants. The results of this study suggest that breastfed infants aged 4-6 months may have a risk of zinc deficiency and that risk is associated with maternal zinc status and breast milk zinc concentrations.

Key Words: zinc deficiency, breastfeeding, breast milk zinc, infant zinc status, lactating women

INTRODUCTION

Zinc is essential for normal growth, development, and immune function of infants.¹⁻³ Infants have hepatic stores of zinc from intrauterine life, which provide zinc for utilization during 4-6 months of life.⁴ After birth, they also obtain zinc from their daily dietary intake.

The World Health Organization has recommended exclusive breastfeeding during the first 6 months of life, and continued breastfeeding with appropriate complementary food until the age of 2 years or later.⁵ Breast milk is a nutritionally complete diet that provides adequate nutrients for infants during the first 6 months.⁶ After this period, complementary feeding has an important role in maintaining the adequacy of nutrients for infants.^{7,8} Breast milk zinc concentration reaches the highest level during the early period of lactation and sharply declines during the first 3 months.⁹ Because of its

low concentration in breast milk after 3 months of lactating period, zinc may be determined as the first-limiting nutrient in breast milk.¹⁰ Due to the limited zinc storage and a physiologic decrease in breast milk zinc concentration, the period of first 4-6 months of life seems to be the transition period during infancy for a high risk of zinc deficiency.

There are several reports on zinc deficiency in infants

Corresponding Author: Dr Nalinee Chongviriyaphan, Division of Nutrition, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 270 Rama VI Road, Ratchathewi, Bangkok 10400, Thailand.

Tel: +66-2-2011776, +66-83-8415556; Fax: +66-2-2011776

Email: nalinee.cho@mahidol.ac.th

Manuscript received 20 March 2014. Initial review completed 03 July 2014. Revision accepted 04 August 2014.

doi: 10.6133/apjcn.2015.24.2.06

from previous studies. In 2006, Wasantwisut et al¹¹ conducted a supplementation trial on exclusively breastfed infants aged 4 months in the northeast area of Thailand. That study showed that the prevalence of zinc deficiency in infants at baseline, determined by serum zinc concentration below 10.7 $\mu\text{mol/L}$, was 45-63%; and mean serum zinc concentrations among different supplemented groups in the study were in the range of 10.4 to 11.7 $\mu\text{mol/L}$. Lind et al¹² reported that 78% of Indonesian infants aged 4-6 months in their study had zinc deficiency. Han et al¹³ studied zinc status of infants in Korea and found that 13% of infant aged 5 months had serum zinc concentration below 9.9 $\mu\text{mol/L}$.

As mentioned earlier, zinc is essential for infants and there are many factors affecting zinc status during infancy, especially during the high-risk period of 4-6 months of life. Therefore, this study was conducted to determine the prevalence of zinc deficiency in infants aged 4-6 months and its associated factors.

MATERIALS AND METHODS

Subjects and study design

This was a cross-sectional study enrolled healthy infants aged between 4 to 6 months and their mothers at the well baby clinic, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The enrolments were done during 2 separate periods of time; September 2009 - March 2010 and January - October 2011. Infants who were born prematurely, having recent illness within 4 weeks before enrolment, and receiving vitamin and/or mineral supplementation were excluded from the study. The study protocol was approved by Committee of Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 06-52-12 and ID 01-54-01). All participants provided written informed consent before enrolment.

Data collection

Demographic data

Demographic data including infant age and sex, maternal age, religion, socioeconomic status, and maternal education level was collected using questionnaires.

Antenatal and perinatal data was reviewed from the medical records in mothers who had antenatal care and delivered their babies at Ramathibodi Hospital. The handbooks of mother-and-child health were used for collecting those data in mothers who attended antenatal care and delivered elsewhere.

Infant feeding data

Feeding data was collected by interviewing caregivers. The data included the types of milk being fed to infants and the introduction of complementary foods. Regarding types of milk being fed, infants were classified into 3 groups as follows; (1) the breastfed (BF) group having infants who were exclusively breastfed or predominantly breastfed, (2) the formula-fed (FF) group for those being fed only infant formula, and (3) the mixed breastfed and formula-fed (MF) group for those fed both breast milk and infant formula.

Anthropometric data

Weight and length measurements were performed using a standard technique by well-trained personnel at the well baby clinic. The body weight of infants, wearing light clothing, was measured to the nearest 1 g using a digital infant scale (Seca model 727). The recumbent length of infants was measured to the nearest 0.1 cm using a wooden board with sliding foot piece. Weight and length data was calculated to Z-score using the INMU-NutriStat software program based on the data of Thai Growth Standard for 0-19 Years, Ministry of Public Health 1997. This software was developed by Institute of Nutrition, Mahidol University.

Sample collection

Non-fasting blood samples were collected via peripheral venepuncture from infants and lactating mothers during 2:00-4:00 p.m. of the visit day. Two mL of blood from each subject was taken and collected in a tube containing EDTA. All blood samples were centrifuged to separate plasma within 3 hours after collection; the plasma samples were then stored at -80°C until analysis. All glassware and plastic wares used in collecting blood samples were treated with HNO_3 overnight to be demineralized before using in order to prevent contamination of zinc from environment.

Lactating mothers who participated in this study during the second period of study (January - October 2011) were asked to collect breast milk samples. The collection of breast milk was done during 2:00-4:00 p.m. on the visit day using an electric breast pump. Nipples and areolas were cleaned with deionized water before milk collection. The first 15-20 mL of breast milk from one side of the breast was obtained. Breast shields, connectors, and collecting chambers were demineralized with HNO_3 before using. Breast milk samples were stored in -80°C until analysis.

Analysis of plasma and breast milk zinc concentrations

Zinc concentration in plasma was analysed using a flame atomic absorption spectrophotometry (GBC Avanta S, GBC Scientific Equipment Pty Ltd., Dandenong, Australia) according to the modified method of Smith et al¹⁴ and Stevens et al.¹⁵ After the proper dilution with deionized water, plasma samples were immediately analysed. Calibration was done against standards using a commercial zinc standard solution (Sigma Chemical Co., St. Louis, MO, USA). The accuracy of determination was assessed by making comparisons with standard reference materials (SeronormTM) obtained from SERO AS (Billingstad, Norway). Zinc deficiency in this study was defined as plasma zinc concentration below 10.7 $\mu\text{mol/L}$.¹⁶

Zinc concentration in breast milk was determined using an inductively coupled plasma mass spectrometry (ICP-MS).¹⁷ Prior to the ICP-MS determination of zinc, breast milk samples were digested using HNO_3 in a closed vessel under microwave radiation (Bergohof Products + Instrument, GmbH, Eningen, Germany). The ICP-MS used in this study was Perkin-Elmer Sciex ELAN6000 model (Shelton, CT, USA).

Statistical analysis

All analyses were conducted using PASW Statistics 18 (SPSS Inc.). The data distribution was tested with Kolmogorov-Smirnov test of normality. Frequencies were used for the prevalence data. Among the data with normal distribution, analysis of variance (ANOVA) was used for analysing the differences between continuous variables. For those not having normal distribution, Kolmogorov-Smirnov test was used. Fisher's exact test was used for a comparison of the proportion. The correlation of data was analysed with Pearson's correlation coefficient. Logistic regression was used for determination of the factors involved with zinc status of infants. Statistical significance was accepted at $p < 0.05$.

RESULTS

There were 176 pairs of infants and mothers enrolled in this study; they were classified into the BF group ($n=54$), the FF group ($n=84$), and the MF group ($n=38$). The demographic data and anthropometric data are shown in Table 1. There was no significant difference in these data among different feeding types. Complementary foods were introduced to 113 infants (64%) in this study. According to infant feeding types, 25 infants from the BF group (46.2%), 65 infants from the FF group (77.3%), and 23 infants from the MF group (60.5%) received complimentary foods. The proportion of infants receiving complementary foods was significantly higher in the FF group compared with the BF group (Odd ratio 4.33, 95% confident interval 2.1-9.1, $p < 0.01$) and the MF group (Odd ratio 2.35, 95% confident interval 1.02-5.42, $p = 0.034$).

Blood samples were collected from 158 infants (89.8%). We were unable to obtain blood samples from 15 infants after 2-3 attempts of peripheral venepuncture; and parents of 3 infants were unwilling to let their infants have blood sampling. Among these 158 infants, 12 infants (7.6%) had plasma zinc concentration below 10.7 $\mu\text{mol/L}$. According to feeding types, the BF group had the highest prevalence of zinc deficiency (14.9%, 7 of 47 infants) compared with the FF group (5.3%, 4 of 75 infants) and the MF group (2.9%, 1 of 35 infants). The prevalence of zinc deficiency in breastfed infants was almost 3 times higher than that in formula-fed infants but this was not statistically significant (Odd ratio 3.15, 95% confident interval 0.86-11.36, $p = 0.071$). Mean plasma zinc concentrations of these 3 groups of infants were not significantly different (18.4 ± 7.6 , 20.0 ± 6.3 , 18.2 ± 4.9

$\mu\text{mol/L}$ for the BF, the FF, and the MF groups, respectively).

Comparing demographic and anthropometric data, there was no significant difference between infants with zinc deficiency and zinc sufficiency (Table 2). There was also no difference in socioeconomic status, maternal and caregiver education levels, and antenatal history between infants with and without zinc deficiency.

Infants who received complementary foods had a comparable mean plasma zinc concentration to those who had no complementary foods (19.5 ± 6.18 vs 18.5 ± 7.01 $\mu\text{mol/L}$, $p = 0.38$). In the BF group, which had the highest prevalence of zinc deficiency, the median plasma zinc concentration was not significantly different between infants with and without complementary foods (17.6 vs 19.1 $\mu\text{mol/L}$, $p = 0.62$). There was no correlation between plasma zinc concentration and the age of infants ($r = -0.045$, $p = 0.76$).

Plasma zinc was determined in 44 of 54 mothers (81.5%) belonging to the BF group. Six mothers in that group (13.6%) had zinc deficiency. We found that infants with zinc deficiency in the BF group had a higher proportion of maternal zinc deficiency than those with zinc sufficiency (66.7% vs 16.2%, odd ratio 2.54, 95% confident interval 0.81-7.86, $p = 0.02$). The median maternal plasma zinc concentration in infants with zinc deficiency in the BF group was significantly lower than that in those with zinc sufficiency (10.0 vs 13.2 $\mu\text{mol/L}$, $p = 0.043$). There was a positive correlation between maternal and infant plasma zinc concentration ($r = 0.287$, $p = 0.013$).

Breast milk samples were collected from 34 mothers whose infants were in the BF and the MF groups; 14 and 20 samples were from mothers of infants aged 4 and 6 months, respectively. The median level of breast milk zinc was 1.57 mg/L with the maximum of 3.2 mg/L and the minimum of 0.5 mg/L. There was no significant difference between the median breast milk zinc concentrations from mothers of infants aged 4 and 6 months (1.52 and 1.61 mg/L, respectively, $p = 0.70$). There was a positive correlation between zinc concentration in infants' plasma and breast milk ($r = 0.62$, $p = 0.001$) and between zinc concentration in plasma of lactating mothers and their breast milk ($r = 0.56$, $p = 0.016$); but no correlation between breast milk zinc concentration and the age of infants was found ($r = 0.057$, $p = 0.81$).

Using a logistic regression model to determine the factors associated with infant zinc status among the

Table 1. Demographic and anthropometric data of infants according to feeding types

Data	BF (n=54)	FF (n=84)	MF (n=38)	p-value
Sex (M/F)	26/28	42/42	19/19	0.975
Age (d, mean \pm SD)	150 \pm 31	155 \pm 31	148 \pm 30	0.473
Maternal age (y, mean \pm SD)	30 \pm 6	30 \pm 6	31 \pm 5	0.593
Gestational age (wk, mean \pm SD)	38.6 \pm 1.1	38.8 \pm 1.2	38.5 \pm 1.2	0.543
Birth weight (g, mean \pm SD)	3,237 \pm 455	3,164 \pm 433	3,098 \pm 465	0.334
Birth length (cm, mean \pm SD)	50.2 \pm 1.9	49.7 \pm 1.8	49.7 \pm 2.2	0.264
Weight-for-age Z-scores	1.27 \pm 1.27	1.00 \pm 2.75	0.79 \pm 1.24	0.559
Length-for-age Z-scores	0.88 \pm 0.11	0.96 \pm 0.10	1.00 \pm 0.16	0.833
Weight-for-length Z-scores	1.11 \pm 0.15	1.19 \pm 0.13	1.15 \pm 0.18	0.165

BF: breastfed group; FF: formula-fed group; MF: mixed breastfed and formula-fed group.

Table 2. Demographic and anthropometric data of infants by zinc status

Data	Zinc sufficiency (n=146) (plasma concentration $\geq 10.7 \mu\text{mol/L}$)	Zinc deficiency (n=12) (plasma concentration $< 10.7 \mu\text{mol/L}$)	p-value
Sex (M/F)	71/75	7/5	0.365
Age (d, mean \pm SD)	151 \pm 31	151 \pm 36	0.959
Maternal age (d, mean \pm SD)	30 \pm 6	31 \pm 5	0.436
Birth weight (g, mean \pm SD)	3,167 \pm 465	3,288 \pm 216	0.112
Weight gain/day (g, mean \pm SD)	27 \pm 6	28 \pm 8	0.666
Length gain/week (cm, mean \pm SD)	0.76 \pm 0.45	0.68 \pm 0.14	0.508
Weight-for-age Z-scores	1.12 \pm 1.30	1.52 \pm 1.12	0.312
Length-for-age Z-scores	0.66 \pm 0.95	0.86 \pm 1.00	0.496
Weight-for-length Z-scores	0.64 \pm 1.17	0.96 \pm 1.24	0.377

Table 3. Factors associated with zinc status in breastfed infants using logistic regression model

Factors	β (SE)	p-value
Maternal zinc concentration	0.60 (0.22)	0.015
Maternal zinc deficiency (yes vs no)	0.14 (3.42)	0.495
Breast milk zinc concentration	0.19 (0.21)	0.315
Complementary foods (yes vs no)	0.11 (2.42)	0.553

breastfed group, maternal plasma zinc concentration was the only factor associated with infant zinc status as shown in Table 3.

DISCUSSION

In the present study, the prevalence of zinc deficiency among healthy infants aged 4-6 months was 7.6%, which was lower than the prevalence of zinc deficiency reported by Wasantwisut et al¹¹, Lind et al¹², and Han et al.¹³ Concerning the prevalence of zinc deficiency among exclusively breastfed Thai infants, we found a lower prevalence than that in the study of Wasantwisut et al. This discrepancy may be due to the differences in the study site, socioeconomic status, eating habit, culture, and the study period. Our study was conducted in Bangkok, the capital city of Thailand; while the other was studied in the rural area in Northeast of Thailand. People in these 2 areas have many factors that are not similar such as preference foods, eating habit, culture, and socioeconomic status; those factors may influence zinc status of infants both during intrauterine life and nursing period.

Regarding the results from this study, the highest prevalence of zinc deficiency was found in exclusively breastfed infants but the mean plasma zinc concentrations were comparable among the 3 groups. Similarly, the study in Korean infants reported by Han et al¹³ showed that mean plasma zinc concentrations of 5-month-old breastfed infants and those who were fed infant formula were not significantly different (13.9 \pm 4.8 and 13.4 \pm 2.5 $\mu\text{mol/L}$, respectively). However, there was no report on the prevalence of zinc deficiency by feeding types in Han's study. Jochum et al¹⁸ reported a study conducted in Germany in 1995 to compare plasma zinc concentrations in 3 groups of 4-month-old infants fed with breast milk, infant formula, and partially hydrolysed whey protein formula (PHF). According to that study, the mean plasma zinc concentration in breastfed infants was 794 $\mu\text{g/L}$ (12.1 $\mu\text{mol/L}$), and significantly higher than that in those fed with infant formula (725 $\mu\text{g/L}$ or 11.1 $\mu\text{mol/L}$) but was comparable to that in the PHF group (807 $\mu\text{g/L}$ or 12.3 $\mu\text{mol/L}$). The mean plasma zinc concentration in

breastfed infants reported by Jochum et al¹⁸ was lower than that found in the present study. In contrast with the present study, formula-fed infants had a significantly lower plasma zinc concentration than breastfed infants. However, the infant formula used at that period of time was not supplemented with zinc. The infant formula used in the study of Jochum et al¹⁸ contained 2.1 mg/L of zinc, while zinc content labelled on the package of infant formulas for infants in our study was 5.6-7.4 mg/L. The PHF used in the study of Jochum et al¹⁸ had zinc content of 5.7 mg/L, which was close to the zinc content in infant formulas in the present study; and plasma zinc concentrations in the PHF group were comparable to those in the breastfed group, which were similar to the results of the present study.

Complementary foods may have an effect on zinc status of infants during 4-6 months old. However, our result showed no difference in plasma zinc concentrations between infants fed with and without complementary foods. Despite there being a higher proportion of infants fed with complementary foods in the FF group than in the BF group, the results from the present study showed that having complementary foods did not affect plasma zinc concentrations in both groups. This may be due to a small amount of complementary foods consumed by infants during this age. In addition, the complementary foods usually introduced in the study area were composed of rice and banana, which are not good sources of dietary zinc. From 113 infants who had started complementary foods in our study, there were only 16 infants (14%) who had red meat or liver in their diets (data is not shown). Nevertheless, the sample size in the present study may be inadequate to reveal an effect of complementary foods. To elucidate this issue, more data on the amount, types, and zinc content of complementary foods as well as a large number of subjects are required.

The median zinc concentration in breast milk in the present study was 1.57 mg/L. Among the studies reported on breast milk zinc concentration during 4-6 months of lactation, the present study reported the similar zinc concentration to that of Jochum et al in Germany¹⁸ (1.4

mg/L), O'Brien et al in USA¹⁹ (1.2 mg/L), Mahdavi et al in Iran²⁰ (1.85 mg/L), but different from those reported by Nakamori et al in Vietnam²¹ (0.59 mg/L) and Yamawaki et al in Japan²² (0.67 mg/L). This difference may be due to the differences in study sites and the population that have different types of foods, eating habits, culture, and socioeconomic status.

We found that zinc concentrations in breast milk were lower than zinc contents in infant formulas. However, zinc is bound to different macromolecules that affect the bioavailability of zinc in breast milk and formula.²³ Zinc in breast milk is more efficiently absorbed than zinc from infant formulas. Lactoferrin in breast milk is suggested to be involved in zinc uptake mechanism.²⁴

Although zinc storage from intrauterine life is supposed to be a source of zinc used during the early period of life, dietary zinc intake has an important role for maintaining zinc status during that period. An infant formula contains a fixed content of zinc, whereas there is a physiological decrease in breast milk zinc concentration during lactating period. It is interesting to consider whether the decrease in breast milk zinc concentration has an effect on zinc status of exclusively breastfed infants aged 4-6 months. In the present study, plasma zinc concentration of breastfed infants had a positive correlation with breast milk zinc concentration. In that case, infants fed breast milk with low zinc concentration will be at risk of zinc deficiency.

We found that 13.6% of lactating mothers had zinc deficiency; and there was a higher proportion of maternal zinc deficiency and lower maternal plasma zinc concentrations in zinc-deficient infants than those in zinc-sufficient ones. Moreover, we found a positive correlation between breast milk zinc concentration and plasma zinc concentrations of mothers. This may imply that maternal zinc status is associated with breast milk zinc concentration.

Zinc is secreted into breast milk via various types of zinc transporters in the mammary gland. The secretion of zinc into breast milk is an active transport process and has a tightly regulated homeostasis.^{25,26} Furthermore, the homeostatic adjustment of zinc metabolism in the body during lactation ensures the adequacy of zinc in breast milk provided to infants.²⁷ Theoretically, zinc concentration in breast milk is independent of maternal zinc status; but the results from previous studies concerning the associations between maternal zinc status, maternal zinc intake, and breast milk zinc concentrations are still controversial. Domellof et al²⁸ compared breast milk zinc concentration in lactating mothers from Sweden and Honduras. In contrast with our study, the unexpected higher breast milk zinc concentrations were found in Honduras mothers who had significantly lower plasma zinc concentrations than that in Swedish mothers. However, the multivariate analysis showed that it was attributed to the difference in milk volume rather than plasma zinc concentrations. A randomized controlled supplementation trial conducted by Kreb et al²⁹, which aimed to determine the effect of zinc supplementation on breast milk zinc concentrations of lactating women in the United States, reported no significant difference in breast milk zinc concentrations. The authors also reported a rate

of decline in breast milk zinc concentrations during 0-9 months of the lactating period between the supplemented and controlled groups, while the zinc-supplemented group had higher plasma zinc concentrations than the controls. The average dietary zinc intake from western diets in the study of Kreb et al was 13 mg/day, suggesting the adequacy of zinc for lactating women. An association of dietary zinc intake and breast milk zinc concentrations was demonstrated by Nakamori et al²¹, who studied breast milk zinc concentrations in Vietnam. They found that almost 60% of lactating women in Vietnam had zinc deficiency and the average dietary zinc intake was 10 mg/day (4 mg below the recommended level for lactating women in Vietnam). There was a positive correlation between dietary zinc intake and breast milk zinc concentration but no correlation between plasma and breast milk zinc concentrations was found. An animal study by Dempsey et al³⁰ showed that milk zinc concentrations in mice fed a zinc-marginal (ZM) diet was 15% lower than those fed a zinc-adequate (ZA) diet even though zinc transporter-2 in the mammary glands of the ZM-diet group was 90% greater than that of the ZA-diet group. Dietary zinc intake may have an effect on breast milk zinc concentrations if it is lower than the threshold of active transport mechanisms in the mammary glands.

Concerning the associations between zinc status and zinc intake of mothers, as well as their breast milk zinc concentrations, the differences in maternal zinc status and dietary zinc intake among countries yield different results. To our knowledge, there is limited evidence to determine the factors associated with breast milk zinc concentrations in Thai lactating women. We need further studies to demonstrate the associations among maternal and infant zinc status, breast milk concentrations, and their dietary intakes as well as to understand the mechanisms involved.

To our knowledge, the present study is the first in Thailand that has reported the prevalence of zinc deficiency in healthy infants aged 4-6 months, classified by infant feeding types and breast milk zinc concentration during 4-6 months of lactation. We can report that maternal plasma zinc was associated with infant zinc status among breastfed group, but cannot identify any factors associated with zinc status of infants in other feeding groups. There are some limitations in our study, including the sample size and a problem of blood sample collection in healthy infants. The sample size might be a significant issue since the different prevalence in zinc status between feeding groups and plasma zinc levels were not statistically significant. In the present study, we found a higher prevalence of zinc deficiency in exclusively breastfed infants than that in other groups of infants. Accordingly, specific issues such as the quality of breast milk with regard to milk composition should be seriously investigated. Moreover, studies on breast milk zinc concentrations are needed to find the factors associated with low breast milk zinc concentrations, as we found that plasma zinc concentrations of infants were associated with zinc concentrations in breast milk. Maternal dietary zinc intake is an interesting issue that may reveal some effects on breast milk or plasma zinc concentrations of lactating mothers. Unfortunately, maternal dietary intake was not analysed in this study.

This information would be important in establishing the anticipatory guidance concerning dietary intake of lactating women in Thailand.

In conclusion, our study revealed that exclusively breastfed infants aged 4-6 months, especially those having maternal zinc deficiency, had a high prevalence of zinc deficiency; and their zinc status was positively correlated with breast milk zinc concentrations. The results of our study suggest that zinc status of infants is associated with maternal zinc status and breast milk zinc concentrations. Factors concerning maternal and infant zinc status, especially in exclusively breastfed infants, as well as breast milk zinc concentration should be further investigated. In addition, further studies should be done to determine whether breast milk from mothers of the population at risk provides an adequate amount of zinc for infants until 6 months of age. It is recommended that breast milk is the best diet for infants and in particular exclusive breastfeeding during the first 6 months of life. Therefore, the quality of breast milk should be the major concern to assure that infants receive adequate nutrients required for their growth and development at full potential. Researchers should be encouraged to conduct more scientific studies on breast milk and to improve the quality of breastfeeding in Thailand.

ACKNOWLEDGEMENT

We would like to express our sincere thanks to all infants and mothers who participated in this study, health care personnel at the well baby clinic and lactation clinic, Faculty of Medicine Ramathibodi Hospital for their good cooperation, and Professor Emeritus Dr Amnuay Thithapandha for his assistance in editing the manuscript.

AUTHOR DISCLOSURES

We declare that there is no conflict of interest. This study was funded by Development potential of Thai people project, Faculty of Medicine Ramathibodi Hospital, Mahidol University.

REFERENCES

- Salgueiro M, Zubillaga MB, Lysionek AE, Caro RA, Weill R, Boccio JR. The role of zinc in the growth and development of children. *Nutrition*. 2002;18:510-9. doi: 10.1016/S0899-9007(01)00812-7.
- Black MM. The evidence linking zinc deficiency with children's cognitive and motor functioning. *J Nutr*. 2003;133:1473S-6S.
- Prasad AS. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp Gerontol*. 2008;43:370-7. doi: 10.1016/j.exger.2007.10.013.
- Krebs NF, Westcott JL, Rodden DJ, Ferguson KW, Miller LV, Hambidge KM. Exchangeable zinc pool size at birth is smaller in small-for-gestational-age than in appropriate-for-gestational-age preterm infants. *Am J Clin Nutr*. 2006;84:1340-3.
- Krama MS, Kakuma R. The optimal duration of breastfeeding: a systematic review. Geneva: World Health Organization; 2002.
- Butte NF, Lopez-Alarcon MG, Garza C. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. Geneva: World Health Organization; 2002.
- Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B et al. ESPGHAN Committee on Nutrition. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr*. 2008;46:99-110.
- Solomons NW, Vossenaar M. Nutrient density in complementary feeding of infants and toddlers. *Eur J Clin Nutr*. 2013;67:501-6. doi: 10.1038/ejcn.2013.46.
- Dorea JG. Zinc in human milk. *Nutr Res*. 2000;20:1645-87. doi: 10.1016/S0271-5317(00)00243-8.
- Dorea JG. Is zinc a first limiting nutrient in human milk? *Nutr Res*. 1993;13:659-66. doi: 10.1016/S0271-5317(05)80558-5.
- Wasantwisut E, Winichagoon P, Chitchumroonchokchai C, Yamborisut U, Boonpradern A, Pongcharoen T, Sranacharoenpong K, Russameesopaphorn W. Iron and zinc supplementation improved iron and zinc status, but not physical growth, of apparently healthy, breast-fed infants in rural communities of northeast Thailand. *J Nutr*. 2006;136:2405-11.
- Lind T, Lönnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekström EC, Persson L. A community-based randomized controlled trial of iron and zinc supplement in Indonesian infants: interactions between iron and zinc. *Am J Clin Nutr*. 2003;77:883-90.
- Han YH, Yon M, Han HS, Johnston KE, Tamura T, Hyun T. Zinc status and growth of Korean infants fed human milk, casein-based, or soy-based formula: three-year longitudinal study. *Nutr Res Pract*. 2011;5:46-51. doi: 10.4162/nrp.2011.5.1.46.
- Smith JC Jr, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. *Clin Chem*. 1979;25:1487-91.
- Stevens MD, Mac Kenzie WF, Anand VD. A simplified method for determination of zinc in whole blood, plasma, and erythrocytes by atomic absorption spectrophotometry. *Biochem Med*. 1977;18:158-63. doi: 10.1016/0006-2944(77)90087-4.
- de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusion of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. *Food Nutr Bull*. 2007;28(Suppl):S480-4.
- McKinstry PJ, Indyk HE, Kim ND. The determination of major and minor elements in milk and infant formula by slurry nebulisation and inductively coupled plasma-optical emission spectrometry (ICP-OES). *Food Chem*. 1999;65:245-52. doi: 10.1016/S0308-8146(98)00183-6.
- Jochum F, Fuchs A, Cser A, Menzel H, Lombeck I. Trace mineral status of full-term infants fed human milk, milk-based formula or partially hydrolysed whey protein formula. *Analyst*. 1995;120:905-9. doi: 10.1039/an952000905.
- O'Brien CE, Krebs NF, Westcott JL, Dong F. Relationships among plasma zinc, plasma prolactin, milk transfer, and milk zinc in lactating women. *J Hum Lact*. 2007;23:179-83. doi: 10.1177/0890334407300021.
- Mahdavi R, Nikniaz L, Gayemmagami SJ. Association between zinc, copper, and iron concentration in breast milk and growth of healthy infants in Tabriz, Iran. *Biol Trace Elem Res*. 2010;135:174-81. doi: 10.1007/s12011-009-8510-y.
- Nakamori M, Ninh NX, Isomura H, Yoshiike N, Hien VT, Nhug BT, Nhien NV, Nakano T, Khan NC, Yamamoto S. Nutritional status of lactating mothers and their breast milk concentration of iron, zinc and copper in rural Vietnam. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55:338-45. doi: 10.3177/jnsv.55.338.
- Yamawaki N, Yamada M, Kan-no T, Kojima T, Kaneko T, Yonekubo A. Macronutrient, mineral and trace element composition of breast milk from Japanese women. *J Trace Elem Med Biol*. 2005;19:171-81. doi: 10.1016/j.jtemb.2005.

- 05.001.
23. Blakeborough P, Salter DN, Gurr MI. Zinc binding in cow's milk and human milk. *Biochem J.* 1983;209:505-12.
24. Sandström B, Cederblad A, Lönnerdal B. Zinc absorption from human milk, cow's milk, and infant formulas. *Am J Dis Child.* 1983;137:726-9. doi: 10.1001/archpedi.1983.02140340010002.
25. Lönnerdal B. Trace element transport in the mammary gland. *Annu Rev Nutr.* 2007;27:165-77. doi: 10.1146/annurev.nutr.27.061406.093809.
26. Kelleher SL, Lönnerdal B. Molecular regulation of milk trace mineral homeostasis. *Mol Aspects Med.* 2005;26:328-39. doi: 10.1016/j.mam.2005.07.005.
27. Donangelo CM, King JC. Maternal zinc intakes and homeostatic adjustments during pregnancy and lactation. *Nutrients.* 2012;4:782-98. doi: 10.3390/nu4070782.
28. Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Hernell O. Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. *Am J Clin Nutr.* 2004;79:1111-5.
29. Krebs NF, Reidinger CJ, Hartley S, Robertson AD, Hambidge KM. Zinc supplementation during lactation: effects on maternal status and milk zinc concentrations. *Am J Clin Nutr.* 1995;61:1030-6.
30. Dempsey C, McCormick NH, Croxford TP, Seo YA, Grider A, Kelleher SL. Marginal maternal zinc deficiency in lactating mice reduces secretory capacity and alters milk composition. *J Nutr.* 2012;142:656-60. doi: 10.3945/jn.111.150623.

Original Article

Maternal zinc status is associated with breast milk zinc concentration and zinc status in breastfed infants aged 4-6 months

Oraporn Dumrongwongsiri MD¹, Umaporn Suthutvoravut MD¹, Suthida Chatvutinun MS², Phanphen Phoonlabdacha MS¹, Areeporn Sangcakul MS³, Artitaya Siripinyanond PhD⁴, Usana Thiengmanee BSc⁴, Nalinee Chongviriyaphan MD, PhD¹

¹Division of Nutrition, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

²Pediatric Nursing Division, Nursing Service Department, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

³Research Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

⁴Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand

母亲锌营养状况与母乳锌浓度和母乳喂养的 4-6 个月婴儿的锌营养状况的关系

母乳为生命的前 6 个月提供了充足的营养素，然而，有些报道称母乳喂养婴儿存在锌缺乏。本研究为确定 4-6 个月婴儿锌缺乏的患病率及相关因素。4-6 个月的健康婴儿和他们的母亲被纳入研究，根据喂养类型将他们分为母乳喂养组（BF）、配方奶喂养组（FF）和混合喂养组（MF）。收集的数据包括人口学资料、围产期资料、喂奶和人体测量资料。收集婴儿和乳母的血样本和乳汁样本来分析血浆和母乳中的锌浓度。158 名婴儿锌缺乏（血浆浓度低于 10.7 mol/L）的发生率为 7.6%。根据喂养方式，BF、FF 和 MF 锌缺乏的发生率分别为 14.9%、5.3%和 2.9%。与没有锌缺乏的母乳喂养儿相比，锌缺乏的母乳喂养儿其母亲的锌浓度显著低。锌缺乏婴儿的母亲锌缺乏比例高于不缺锌婴儿的母亲（66.7%比 16.2%， $p=0.02$ ）。母乳中的锌浓度与婴儿（ $r=0.62$ ， $p=0.01$ ）和乳母（ $r=0.65$ ， $p=0.016$ ）血浆中的锌浓度呈正相关。回归分析发现母乳喂养的婴儿锌营养状况与乳母血浆锌浓度相关。本研究表明母乳喂养的 4-6 个月婴儿存在锌缺乏风险，这种风险与乳母锌营养状况和乳汁锌浓度有关。

关键词：锌缺乏、母乳喂养、母乳锌、婴儿锌状态、哺乳期妇女