Zinc Supplementation does not Affect the Breast Milk Zinc Concentration of Lactating Women Belonging to Low Socioeconomic Population

Sunil Sazawal1,2, Robert E. Black1, Pratibha Dhingra3, Sanju Jalla3, Nancy Krebs3, Pooja Malik1, Usha Dhingra1 and Maharaj K. Bhan4

1Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, USA
2Center for Micronutrient Research, Department of Biochemistry, Annamalai University, India
3Section of Nutrition, Department of Pediatrics, University of Colorado Health Sciences Center, USA
4Department of Biotechnology, Block-2, 7th Floor C.G.O. Complex, Lodi Road, India

INTRODUCTION

In the last decade there has been a progressive increase in understanding of the occurrence of zinc deficiency in infants and preschool children and its impact on morbidity, growth and mortality [1,2]. Zinc deficiency may occur early in life, especially among low birth weight infants. Joint WHO/IAEA collaborative study reports that zinc body storages are not extensive and it should be taken in adequate quantities for optimal growth and development [3]. Reports confirmed positive effects of zinc supplements in low birth weight infants in reducing morbidity and mortality in the first year of life [4-6]. Breast milk zinc is an important source of bio-available zinc for infants, however, the zinc concentration in breast milk declines at a faster rate than other minerals, with increasing postpartum duration of breastfeeding [6,7]. Studies of breast milk zinc concentrations in developing countries have yielded inconsistent results in relation to the impact of zinc supplementation in lactating mothers. This might be due to high dietary zinc intakes and normal zinc nutriture of women in these settings [9-11]. Krebs et al. [12] reported that a mean threshold intake of 13 mg/d dietary zinc during lactation appears to be necessary to ensure that milk zinc concentrations are adequate. Thus, zinc supplementation could potentially influence the breast milk composition [13]. This might offer an ideal route to provide additional zinc to the infant during the first 6 months of life in populations with low zinc intake.

Abstract

Background: Zinc concentrations of breast milk among lactating women are less affected through zinc supplementation in developed countries, but may potentially affect lactating women with suboptimal zinc status from developing countries.

Objective: In a randomized, double-blind trial, to evaluate the effect of zinc supplementation on breast milk zinc concentrations of lactating women with suboptimal zinc status.

Methods: Lactating women 0-2 mo and 4-6 mo postpartum were randomly allocated to receive either multivitamins plus zinc (40mg) daily in 2 divided doses (n=50, 0-2 mo; n=51, 4-6 mo) or same supplement without zinc (n=61, 0-2 mo; n=50, 4-6 mo) for 1 month. A mid-feeding 10ml breast milk sample was collected at base- and end-line.

Results: Following zinc supplementation, change in breast milk zinc concentrations (μmol/L) between the zinc plus multivitamins group (ZMV) and multivitamin group (MV) of lactating women both at 0-2 months (ZMV: -18.9 ± 28.2, MV: -22.1 ± 16.1) and 4-6 months postpartum (ZMV: -2.2 ± 6.6, MV: -0.6 ± 9.5) was similar. Among 0-2 months postpartum women with a low baseline breast milk zinc concentration, zinc supplementation showed a non-significant rate of decline in breast milk zinc concentration (ZMV: 1.4 ± 19.3, MV: 9.7 ± 8.5, p<0.059), while in 4-6 months postpartum women, differences were not significant.

Conclusion: Zinc supplementation only affected a subgroup with low breast milk zinc concentrations in early lactation. Breast milk zinc concentrations seem to be maintained by active transport, unaffected by zinc supplementation in a population of low zinc intake.
dietary intake of zinc. We conducted a double-blind randomized controlled trial to evaluate if zinc supplementation could affect breast milk zinc concentrations of lactating women, with suboptimal zinc status in a developing country.

METHODS
Study participants

In the peri-urban New Delhi, during April 1994 and July 1994, 212 women, who were breastfeeding either 0-2 months old or 4-6 months old infants, who were likely to stay in the area for the next 1 month, not suffering from any chronic systemic illness or severe anemia, not requiring vitamin or micronutrient prescription, and providing consent to participate, were enrolled. The design of the trial is described in (Figure 1). The study was approved by the Committee for Human Research review at the All India Institute of Medical Sciences.

Enrollment and randomization

Two separate lists using randomization with permuted blocks of fixed length of 10 were drawn, one for lactating women 0-2 months postpartum and the other for women 4-6 months postpartum. These lists provided one of 8 letter codes against each serial number. Four letter codes belonged to each treatment group and the supplement bottles were labeled with 8 letter codes. Enrolled lactating women in two strata i.e. 0-2 months (n=111) and 4-6 months (n=101) postpartum were given a serial number which was used to allocate them to receive either multivitamins plus 40 mg zinc/day as zinc gluconate or an identical control supplement (multivitamins without zinc). The allocation code against each serial number was available to the pharmacy assistant who checked allocation code of the women, and then labeled the bottle with her name and identification information. In the field, the supplement was identified by name and identification of women and not by code. Neither participant nor investigator or field team had any knowledge regarding the treatment group. The actual allocation code of 8 letters was with the Sandoz India Ltd (Mumbai, India) and statistician in Baltimore.

For the baseline assessment, a nutritionist visited women at home, where the purpose of the study was explained and verbal consent obtained. Once the women were enrolled, the information regarding the date of delivery, frequency of consumption of foods especially rich in zinc, feeding information of the child was gathered and a mid-feeding 10 ml breast milk sample was collected.

Each woman was given an enrollment number corresponding to the serial number allocated, one bottle containing 250 ml supplement was given to be kept at home and replaced after 12 days. Enrolled women were visited by the field assistant daily and were given the supplement once each day in the morning; the second measured dose was left for the women to take in the evening. A daily record of the consumption of previous day’s zinc supplement was maintained. At the end of the 30 days of supplementation, the study nutritionist again visited the women to obtain a second breast milk sample. During the daily visitation, the occurrences of vomiting, diarrhea, rash, or any stomach discomfort (gastric irritation) were recorded.

At baseline on a random sub-sample of 20 women, dietary intake was assessed using 24-h dietary recall method [14]. The intake of energy and zinc was calculated by using the Nutritive Value of Indian Foods [15].

Power estimation

We estimated sample size (β - 0.9, α - 0.05 two sided) to detect a shift in distribution of breast milk zinc concentrations with overlap of 10% to baseline values. This amounted to a difference of 30% in 0-2 month postpartum and 25% in 4-6 month postpartum lactating women in their breast milk zinc levels with one month zinc supplementation. Given the actual values and participants, the study had a power of 80% in 0-2 month strata and 85% in 4-6 month strata for targeted reductions.

Intervention

The supplement was a liquid preparation manufactured by Sandoz India Ltd (Mumbai, India). Each 10 ml of the supplement contained vitamin A (480 µg), thiamine (1.2 mg), riboflavin (1.0 mg), pyridoxine (1.0 mg), cholecalciferol (5.0 µg), alpha tocopherol (6.0 mg) and niacinamide (20 mg). The treatment preparation in addition contained zinc as zinc gluconate (20 mg of elemental zinc per 10 ml). Women were given 10 ml of the preparation twice daily for 30 days. The dose received was equivalent to 40 mg zinc/day. The supplements were identical in taste, color and packaging.

Method for breast milk sample collection and processing

A standard procedure was used for collection of breast milk samples for zinc analysis [16]. The nipple and areola of the enrolled women and the hands of the collector were washed and rinsed with double distilled water. Milk was manually expressed from each breast directly into the zinc-free polypropylene vials.
Central intake foods (Table 1). The reported dietary intake of zinc in 0-2 months postpartum varied between 1-6.5 mg/d with a mean of 4.5 mg/d and in 4-6 mo postpartum it varied between 4-6 mg/d with a mean of 5.5 mg/d. Intake of dietary zinc among this population is far below the recommended dietary allowances for lactating women [18] and the reported results from the US studies [12,19] (Figure 2).

There was a marked decline in the breast milk zinc concentrations from baseline to end of study in both the groups (ZMV: paired diff -10.5 ± 21.9 µmol/ L, p<0.001; MV: paired diff -12.4 ± 17.2 µmol/ L, p<0.001), but this difference was not statistically significant between the two groups (ZMV and MV, p=0.47). When the paired difference in breast milk zinc concentrations between the two groups (ZMV and MV) was evaluated by postpartum duration i.e. 0-2 months and 4-6 months postpartum, no significant differences were observed (Table 2).

In order to explore if the effect of zinc was related to baseline breast milk zinc concentrations, women were categorized into two groups according to the baseline breast milk zinc concentration. The threshold concentration was set at 45 µmol/L for 0-2 months (≤50th percentile) and 15.5 mol/L for 4-6 months (≤50th percentile). At 0-2 months, the decline of the breast milk zinc concentration among women with low baseline levels was more in the MV group (-9.7 ± 8.5 µmol/L) as compared to ZMV group (-1.4 ± 19.5 µmol/L, p=0.059) which was of borderline significance. At 4-6 months, there was no statistical difference in the decline of zinc concentration by the baseline zinc concentration in both the groups (Table 3).

The decline was greater in the ZMV group but none of these women had a comparable high frequency of low zinc intake foods (Table 1). The reported dietary intake of zinc in 0-2

### Table 1: Baseline Comparison of Participants in the Studya.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>0-2 month postpartum</th>
<th>4-6 month postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZMV</td>
<td>MV</td>
</tr>
<tr>
<td>Women, n</td>
<td>50</td>
<td>61</td>
</tr>
<tr>
<td>Age of women, y</td>
<td>24.8±5.3</td>
<td>24.6±4.3</td>
</tr>
<tr>
<td>Duration of lactation, %</td>
<td>60.0b</td>
<td>63.9b</td>
</tr>
<tr>
<td>Exclusive breastfeeding, %</td>
<td>12.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Partial breastfeeding, %</td>
<td>88.0</td>
<td>82.0</td>
</tr>
<tr>
<td>Height of women, cm</td>
<td>149.2±5.1</td>
<td>151.1±4.9</td>
</tr>
<tr>
<td>More than 3 children &lt;5 years of age, %</td>
<td>16.0</td>
<td>11.5</td>
</tr>
<tr>
<td>Cumulative no. of times food eaten/month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very rich in zinca</td>
<td>27.9</td>
<td>30.5</td>
</tr>
<tr>
<td>Rich in zincb</td>
<td>19.7</td>
<td>22.8</td>
</tr>
<tr>
<td>Poor in zincc</td>
<td>89.9</td>
<td>99.4</td>
</tr>
<tr>
<td>Very Poor in zincd</td>
<td>121.8</td>
<td>127.5</td>
</tr>
</tbody>
</table>

a Values are mean ± SD or percent
b % of women in 0-30 days postpartum
c % of women in 120-150 days postpartum
d Reference [17]
A small net benefit resulted in terms of zinc delivery to the infants of women having low zinc status/low breast milk zinc concentrations in the early stages of lactation i.e. at 0-2 months postpartum. These results are consistent with cross-sectional and longitudinal trials of lactating women from developed countries [12,22-25] and a trial reported from the developing country [13]. Among women in the 0-2 month postpartum stratum, with baseline milk zinc concentrations below 45 µmol/L, there appeared to be a decrease in the rate of decline of breast milk zinc concentrations with zinc supplementation. Krebs et al, 1985 [20] in a non-randomized trial of maternal zinc supplementation found a slower rate of decline in milk zinc concentrations, but these results were not confirmed in a randomized intervention study by Krebs et al. [12]. Salmenpera et al. [26] reported an elevation of maternal serum zinc concentration during the first 2 months when zinc was supplemented at 40 mg/d and smaller decline in breast milk zinc concentration by 6 month, though not observed with 20 mg zinc supplementation. In a study by Karra et al. [19] zinc supplementation with 25 mg/day showed a positive correlation with breast milk zinc concentration in American women compared with non-supplemented American and Egyptian women. As the study was not a randomized trial the results could have been confounded by other factors. Animal studies, however, suggest that zinc absorption is increased during lactation with the relative increase in dietary zinc intake [27], but not reflected in breast milk zinc concentration with zinc supplementation. In agreement with previous studies breast milk zinc concentrations are not influenced by maternal dietary zinc intake [12,21,22,28-30]. Lack of effect of supplementation indicated in this study, could unlikely be related to low comparisons were significantly different between ZMV and MV groups (Table 4).

Median breast milk zinc concentration at each month of lactation using baseline concentrations of both groups and post-supplementation concentrations of the MV group are presented in Figure 3, in comparison with concentrations reported from Denver [20] and Bangladesh [21]. Zinc concentration declined very rapidly up to 3-4 months postpartum ([µmol/L], 62.8 (1mo), 32.2 (2 mo), 23.1(3 mo), 16.3 (4 mo), 18.3 (5 mo), 18.5 (6 mo), 16.0 (7 mo)), but the decline and concentrations in the study sample were similar to the US and Bangladesh populations.

**DISCUSSION**

In a population with low zinc intakes, daily supplementation with 20 mg zinc twice a day, for one month did not alter the decline in breast milk zinc concentrations as lactation progressed. A small net benefit resulted in terms of zinc delivery to the infants of women having low zinc status/low breast milk zinc concentrations in the early stages of lactation i.e. at 0-2 months postpartum. These results are consistent with cross-sectional and longitudinal trials of lactating women from developed countries [12,22-25] and a trial reported from the developing country [13]. Among women in the 0-2 month postpartum stratum, with baseline milk zinc concentrations below 45 µmol/L, there appeared to be a decrease in the rate of decline of breast milk zinc concentrations with zinc supplementation. Krebs et al, 1985...
bioavailability of zinc supplement, as this source of zinc has shown significant increases in plasma zinc after supplementation in children in our previous studies [31].

Limitations of the present study include; diets of all the subjects were not adequately evaluated, maternal serum zinc concentrations were not analyzed and pre-post concentrations of zinc in breast milk were measured only from the single sample collected i.e. at the beginning and end of the study. The association of maternal zinc status with the breast milk zinc concentrations could not be adequately evaluated.

There have been reports of differences in breast milk zinc concentrations by socio-economic status [32] within a study population and lower concentrations in some populations with lower socio-economic status as compared to western population [33]. Our findings and similar findings from China [34] do not exclude that variations may be related to dietary or non-dietary factors other than zinc intake. In this context our finding of a marginal change in breast milk zinc concentration in women with lower values may have some importance, but needs to be confirmed. In the present study, all the necessary precautions were taken while sampling and handling of breast milk samples, therefore, it is unlikely that this could have affected our results.

However, there is data suggesting that at least in part homeostatic mechanisms in response to lower zinc intakes increase fractional absorption and intestinal conservation of endogenous zinc [34-36] and the increase in absorbed zinc may be substantial relative to the output in milk. Although in the present study, it does not get reflected in breast milk zinc concentration. Breast milk zinc concentrations at different months of lactation being comparable to lactating women in developed countries suggests there is an active transport mechanism regulating the concentration of zinc in breast milk, favoring the baby irrespective of the zinc status of the mother. This mechanism it seems does not allow for extra zinc secretion in milk irrespective of excessive maternal zinc intake. The active transport may be due to excretion of zinc via an active pump mechanism [34,37]. More studies are required to fully understand the mechanisms of zinc secretion in breast milk, implications of this secretion for maternal nutrition and the possibility of enhancing breast milk zinc concentrations.

CONCLUSION

Breast milk zinc, an important source of bio-available zinc for infants shows a faster rate of decline postpartum. In developed countries, breast milk zinc concentrations have yielded inconsistent results with maternal zinc supplementation; data from less developing countries is lacking. In the present study when we evaluated the effect of zinc supplementation (20 mg daily for 1 month) on breast milk zinc concentrations of lactating women with suboptimal zinc status, it did not alter the decline in breast milk zinc concentrations as the lactation progressed which was very similar to the US and Bangladesh populations. However, it appears that zinc supplementation affected a subgroup of women with low breast milk zinc concentrations in early lactation, 0-2 months postpartum. The results of this study highlight that maternal zinc supplementation during lactation cannot be used to improve the zinc status of the young infant.

ACKNOWLEDGEMENTS

All the investigators contributed substantially to the design, implementation of the study and to analysis and writing of the paper. We are grateful to the mothers for their participation, time and co-operation.

FUNDING

The study is supported in part from the grants of World Health Organization and Indian Council of Medical Research.

ETHICAL APPROVAL

The study was approved by the Committee for Human Research review at the All India Institute of Medical Sciences. All the procedures were followed according to the ethical standards of the review board.

REFERENCES


Cite this article