

## Trace Element Concentrations in Breast Milk and Sera: Relations With Lactation

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### ABSTRACT

Breast milk has unique properties; one is the variability of the compounds in ratio according to the baby's needs for every lactation period. Iron and zinc are essential elements for life. The aim of this study is to determine Fe and Zn levels in plasma and breast milk of mothers through the first 4 months after delivery and to evaluate its relation with infants' growth and immune system functions. The study conducted through January 2000 – April 2001 in Bursa with 27 mother-infant couples. The babies grouped into two: Group 1 (n: 16) was formed by the breast milk fed babies, and Group 2 (n: 11) included the babies fed with both breast milk and formula. Besides clinical assessment of the babies, laboratory evaluations were performed on the sera of couples and breast milk. While the comparison of Zn level in colostrum with mature and late mature breast milk demonstrated a statistical significance ( $p < 0.05$ ), Fe had a similar trend without significance. Fe deficiency anemia was found in high percentages in both groups; 32.5% of Group1 and 27.7% of Group2 ( $p > 0.05$ ). Our study affirms the suggestion for Fe supplementation for healthy, term infants beginning from 4<sup>th</sup> month regardless of nutritional model.

**Key words:** Breast milk, iron, zinc, lactation period, anemia, immune system, growth.

**Abbreviations:** Appropriate for Gestational Age (AGA); Complete Blood Count (CBC); Iron Binding Capacity (FeBC); Transferrin Saturation (TS); Iron (Fe); Zinc (Zn)

### INTRODUCTION

Breast milk is known to be the best nutrient for the first 6 months of life (Mills, 1990). It has unique properties; one is the variability of the compounds in ratio according to the baby's needs for every lactation period. The recent studies revealed that the levels of essential micronutrients as Fe and Zn in breast milk were decreased through the first months of life (Figure 1). These elements are found at higher levels in cow milk when compared with breast milk however the elements in breast milk are more bioavailable. Therefore, the iron content of breast milk has given credence to protect infants against severe anemia for the first 4 months (Feeley et al 1983; Schramel et al 1988; Nagra 1989).

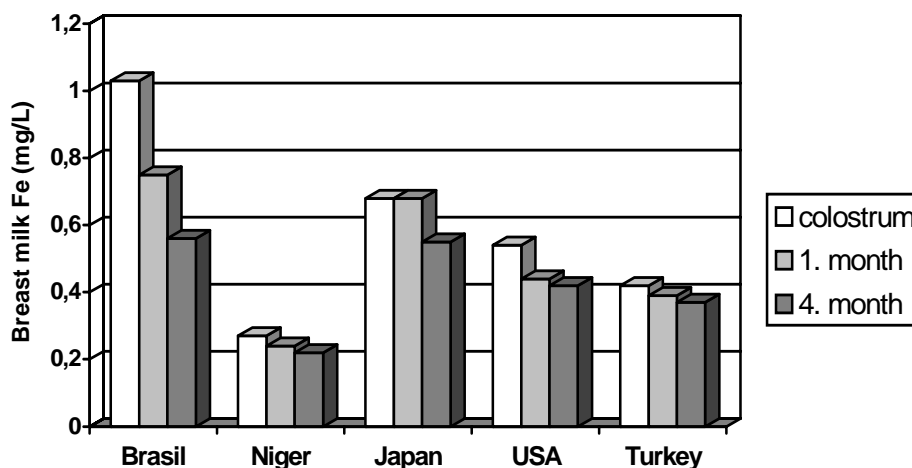


Figure 1. Fe levels in breast milk during lactation period in different countries (Jose 2000)

The results of the surveillance studies demonstrated that approximately 22% of children less than 2 years suffered from anemia due to iron deficiency with an increasing prevalence among cow milk fed infants. Inadequate intake of iron during infancy leads to impaired cognitive development, and zinc deficiency is associated with acrodermatitis, failure to thrive, irritability, impaired neurological functions, and intractable

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Fe deficiency anemia. Both iron and zinc are essential for immune system maturation, T lymphocyte functions, and cytokine synthesis (Shankar and Prasad 1998; Thibault et al 1993).

The aim of this study is to determine Fe and Zn levels in plasma and breast milk of mothers through the first 4 months after delivery and to evaluate its relation with infants' growth and immune system functions.

## MATERIALS AND METHODS

A prospective mother-infant study was conducted between January 2000 – April 2001 in Uludag University Hospital, Bursa State Hospital Obstetrics Department, and Bursa Maternity Hospital. Twenty-seven mothers without a systemic disease or a history of complication during pregnancy and their term, singleton, healthy and, appropriate for gestational age (AGA) infants were considered eligible for the study. The babies grouped into two: Group 1 (n: 16; 8 girls, 8 boys) was formed by the breast milk fed babies, and Group 2 (n: 11; 5 girls, 6 boys) included the babies fed with both breast milk and formula. The clinical evaluations of the babies were done at birth, on the 1<sup>st</sup> and 4<sup>th</sup> months, also weight, height and head circumferences were recorded.

Breast milk were collected from all mothers on the 1-4 days (colostrum), 30-35 days (mature breast milk), and 120-125 days (late mature breast milk) in the morning hours before the babies were fed. Blood samples were obtained from the mothers concurrently with breast milk and from the babies only once, on the 4<sup>th</sup> month. The sera of mothers and babies were used for complete blood count (CBC), determination of serum iron (Fe) and zinc (Zn) levels, transferrin saturation (TS) and iron binding capacity (FeBC). Fe and Zn levels were measured in breast milk also. CBC was performed with impedance-based analyzer (ABBOTT, CELL-DYN<sup>®</sup> 3700, USA). Atomic Absorption Photometry (Shimadzu<sup>™</sup> AA-680 Atomic Absorption Photometry) method was used for measurement of Fe and Zn levels in both serum and breast milk. For the procedure, samples were stored at -20 °C until evaluation.

The blood samples of the babies obtained on the 4<sup>th</sup> month and of the mothers on the first days after delivery were used for determination of lymphocyte subsets (CD3+, CD4+, CD8+, NK) by using flow cytometric method (Immunotech/Coulter<sup>™</sup>).

Anemia was defined as Hb < 12 g/dL and TS < 14% for the mothers, and Hb < 11 g/dL and TS < 12% for the babies (De Maeyer et al, 1989).

The statistical analysis of data was performed with SPSS<sup>™</sup> 13.0 for Windows. Student t test was used for the comparison of weight, height, and head circumferences of the babies. The relations between serum Zn, Fe, FeBC, TS, Hb levels, and lymphocyte subsets were evaluated by using Mann-Whitney U test. The comparison of lymphocyte subsets with Fe levels in serum and breast milk was examined with Pearson correlation rank test. The threshold of significance was set at  $p < 0.05$ .

## RESULTS

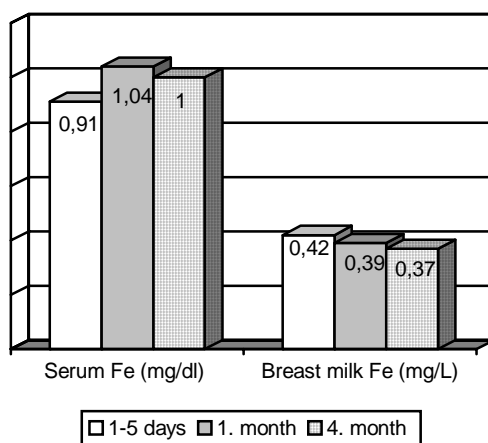
The study was conducted with totally 27 mother-baby couples. While 16 babies fed with only breast milk (Group1), other 11 babies fed mainly with formula but also breast milk (Group2).

The evaluation of breast milk revealed that Fe content of colostrum was  $0.42 \pm 0.15$  mg/L. With a tendency to decrease, it was  $0.39 \pm 0.13$  mg/L on the 1<sup>st</sup> month and exhibited the least level ( $0.37 \pm 0.13$  mg/L) on the 4<sup>th</sup> month. The statistical analyze of Fe levels in breast milk obtained on three different lactation periods was not significant ( $p > 0.05$ ) (Figure 2).

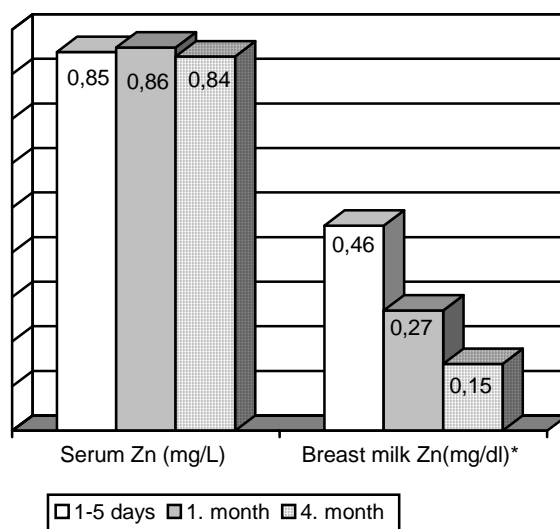
The breast milk Zn levels throughout the study also had a similar trend as Fe, but the comparison of Zn level in colostrum with the levels measured on the 1<sup>st</sup> and 4<sup>th</sup> months demonstrated a statistical significance ( $p < 0.05$ ). The Zn levels of maternal sera did not reveal any significant difference during the study ( $p > 0.05$ ) (Figure 3).

The results of babies showed that serum transferrin saturation on the 4<sup>th</sup> month was higher in Group 2 than in Group1 without a statistical significance ( $p > 0.05$ ) and hemoglobin levels were similar in both groups. Fe deficiency anemia was found in high percentages in groups, 32.5% of Group1 and 27.7% of Group2 ( $p > 0.05$ ).

In addition, serum Zn levels in Group 1 and 2 were not significantly different on the 4<sup>th</sup> month (Table 1).



**Figure 2.** The change in maternal serum and breast milk Fe levels through the first months after birth



**Figure 3.** The change in maternal serum and breast milk Zn levels through the first months after birth.\* $p < 0.05$

**Table 1.** Serum Fe, FeBC, TS, Hb and Zn levels of Group 1 and 2 (Values indicate "mean $\pm$ SD").

	Group1 n:16	Group2 n:11	<i>p</i>
<b>Fe (mg/dL)</b>	0.68 $\pm$ 0.26	0.75 $\pm$ 0.24	>0.05
<b>FeBC(mcg/dL)</b>	372.3 $\pm$ 36.5	360.9 $\pm$ 71.7	>0.05
<b>TS(%)</b>	18.5 $\pm$ 8.6	21.7 $\pm$ 9.1	>0.05
<b>Hb(g/dL)</b>	10.9 $\pm$ 0.8	10.9 $\pm$ 0.7	>0.05
<b>Anemia %</b>	32.5	27.7	>0.05
<b>Zn(mg/dL)</b>	0.85 $\pm$ 0.16	0.83 $\pm$ 0.23	>0.05

On the 4<sup>th</sup> month's evaluation the percentage of lymphocyte subsets in both groups were similar and in normal ranges for age. (Table2).

**Table 2.** Lymphocyte subsets of babies and mothers (Values indicate “mean±SD”)

	<b>Group 1 n:16</b>	<b>Group2 n:11</b>	<b>Mothers n:27</b>	<b>P*</b>
<b>CD3+</b>	%63.2± 5.3	%66.2± 5	%76.2± 5	>0.05
<b>CD4+</b>	%43.6 ±7.8	%46.9± 5.7	%45.8± 9	>0.05
<b>CD8+</b>	%20.9± 6.5	%20.2± 5.2	%30.7± 7	>0.05
<b>CD56+</b>	%7.3± 4.7	%6.7± 2	%8.2± 1.5	>0.05

\*p value between Group 1 and Group 2.

The growth rate of the babies according to weight-height and head circumference charts was similar in both groups and remained parallel to 75<sup>th</sup> percentile throughout the study (Table 3).

**Table 3.** The comparison of groups in weight, height and head circumferences at birth to 4th month. (Values indicate “mean±SD”)

	<b>Group 1 (n:16)</b>		<b>Group 2 (n:11)</b>		<b>p</b>
	<b>Birth</b>	<b>4. month</b>	<b>Birth</b>	<b>4. month</b>	
<b>Weight (kg)</b>	3.3±0.56	6.1±0.15	3.4±0.27	6.9±0.48	>0.05
<b>Height(cm)</b>	49.9±1.7	62.7±2.8	50.6±1.4	65.1±1.6	>0.05
<b>Head circumference (cm)</b>	34.5±1.5	41.2±1.2	34.5±1.0	41.5±1.2	>0.05

## DISCUSSION

Breast milk is the natural, unique, and complete nutrient for the first 6 months of life. Breast milk is poor for most of the minerals but has more bioavailability when compared to cow milk. In addition, it is clear that breast milk concentration of some essential elements as Fe and Zn decreases throughout the lactation period while daily breast milk production is increasing (Ogra and Green 1982; Feeley et al 1983; Emmett and Rogers 1997; Nagra 1989). In the present study, serum Fe levels of mothers were lower on the 4<sup>th</sup> month than the 1<sup>st</sup> month without a statistical significance.

It is known that breast milk Fe and Zn levels are not correlated with nutritional intake and serum levels of mothers (Chierici et al 1999; Arnaud et al 1993). In a study, Franson et al (1984) reported that the serum Fe levels of Ethiopian mothers, who were given iron rich diet, were 0.47±0.19 mg/L. This result was similar to our results (0.46±0.25) although most of the mothers in our study were in low socioeconomic status and did not get nutritional support during pregnancy. Recent studies from Italy and Japan also demonstrated similar results as ours (Cherici et al 1999; Matsuda et al 1984). Consequently, the lack of relation between the maternal Fe-Zn intake and the amounts of these elements in breast milk seems to be accepted.

Bioavailability of Fe in breast milk is high that it provides adequate Fe and prevents anemia during the first 4 months of life (Mills 1990; Calvo et al 1992; Pizarro et al 1991). Contrarily formula feeding, gives rise to anemia due to the cow milk content. In the presented study, the frequency of Fe deficiency anemia in Group 1 and Group 2 was similar. This may be a result of breast milk consumption besides formula and the enhancement of Fe absorption in the intestinal tractus by lactoferrin providing a prophylaxis for anemia in Group 2 who received breast milk and formula together.

Despite the study was conducted in a small group, the development of Fe deficiency in high percentage of babies was emphasizing for the importance of starting Fe supplementation at the 4<sup>th</sup> month.

The Zn depletion is rare in breast fed infants due to the adequate of Zn content of breast milk and its high bioavailability. Sievers et al declared that 2-3mg/dL Zn intake was adequate for infants. In the present study, the Zn content of breast milk was 1.57±1.0 mg/dL at the 4<sup>th</sup> month, and Zn depletion was not found. The bioavailability of Zn in breast milk is high, so the serum Zn levels are expected to be higher in infants who received breast milk (Vigi et al 1984; Sandstrom et al 1983). Our results did not reveal a difference between the serum Zn levels of two groups, and this may be related to the combined feeding of the infants in Group2.

The immune system of infants is immature and breast milk provides a protection for infants against infections by active T lymphocyte and maternal antibody content (Goldman et al 1982; 1996; 1998). In the present study the effect of breast milk on immune system of infants was evaluated on the 4<sup>th</sup> month. The ratio

of T lymphocyte subsets was in normal ranges and similar in both groups and none of the babies had severe bacterial infection. This result might not reflect a reliable data so the immune functions of the babies should be evaluated in further investigations.

The growth assessment of the babies in Group 1 and 2 did not reveal a significant difference but the weight gain of infants in Group 2 during 4 month was more than Group 1. This is considered as a supportive result for the preventive effect of breast milk against adulthood obesity (Dewey et al 1993; Koletzko 2006).

Consequently, the Fe and Zn content of breast milk is altered during the lactation period. Zinc deficiency is seen very rarely in infancy, but anemia due to Fe deficiency occurs in infants about 4 month. While formula feeding leads to Fe deficiency anemia during infancy due to the cow milk content, in the present study high bioavailability of Fe in breast milk plays a preventive role for Group 2. The present study suggests that the babies fed with combination of formula with breast milk also take the advantage of prevention against apparent anemia during the first 4 months of life. Our study affirms the suggestion for Fe supplementation for healthy, term infants beginning from 4<sup>th</sup> month regardless of nutritional model.

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