

Correlation of maternal nutritional status with breast milk content of iron, zinc and vitamin A in rural southern Ethiopia

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Abstract

Background: Malnutrition in women can be categorized as the macro-and micro-nutrient malnutrition. Many women, particularly those living in developing countries like Ethiopia, are underweight and/or stunted. Nutritional status of mothers in Ethiopia is currently a major public health concern. There are three key factors of maternal nutrition that could have an impact on human milk composition: current dietary intake, nutrient store, and alterations in nutrient utilization.

Objective: The aim of this research was to study the relation between maternal nutritional status and contents iron, zinc and vitamin A in breast milk of mothers in a rural community in Southern Ethiopia.

Methods: Community-based cross sectional study was conducted from April to May 2012. Maternal anthropometric status, plasma level of ferritin, iron, zinc and retinol and breast milk composition was assessed. Energy and nutrient intakes were calculated using 24h recall method.

Results: The prevalence of iron deficiency anemia (using hemoglobin concentration), zinc deficiency (using plasma zinc) and vitamin A deficiency (using plasma retinol) among lactating mothers of 6 to 12 months of postpartum in Boricha, SNNP, district were 36%, 100% and 7.3%, respectively. The median calorie and nutrient intake of the lactating mothers were below the Estimated Average Requirement. Dietary intake of vitamin A (beta-carotene) showed a significant positive correlation ($p<0.05$, $r=0.24$) with plasma retinol concentration of lactating mothers. The plasma concentration of retinol also showed a significant correlation ($p<0.05$, $r=0.23$) with that of breast milk retinol concentration.

Conclusion: Maternal malnutrition is a serious problem in the study area. Maternal plasma retinol status was positively related with maternal intake of vitamin A and breast milk retinol concentration..

Key Words: Breast milk composition, dietary intake, plasma nutrient level, Boricha.

Introduction

Many women living in developing countries experience various biological and social stresses that increase the risk of malnutrition throughout life, such as food insecurity, inadequate dietary intake, recurrent infection, frequent parasites, poor healthcare, heavy work burden, and gender inequality (UNICEF, 2009). Malnutrition in women manifests itself both at the macronutrient and micronutrient levels, and is observed by inadequate weight and/or height. Deficiencies in micronutrients such as iron, zinc, and vitamin A are highly prevalent in many regions of the world (USAID and AED 2004).

The nutritional status of mothers in Ethiopia is currently a major public health concern. About 27% of Ethiopian mothers of childbearing age suffer from chronic energy malnutrition with a body mass index (BMI) less than 18.5 and 17% of women aged 15-49 years are anaemic, with 13% mildly anaemic, 3% moderately anaemic, and less than 1% severely anaemic (EDHS 2012). Malnutrition compounded by anaemia, contributes to low birth weight and high mortality, and Ethiopia is plagued by high rates of

each, 11% and 88/1,000 live births, respectively (EDHS 2012). Iron deficiency is the most important cause of nutritional anaemia and is the most common micronutrient deficiency worldwide; which leads to impairment of health, growth, and development, affecting learning ability and thus scholarly achievements. Iron supplementation is currently the most important tool for combating iron deficiency. However, intake of iron supplements in malaria endemic regions among children who are not iron deficient could lead to increased morbidity and possibly mortality (Samuel and Tom 2010). Zinc deficiency increases incidence and severity of infection, impaired growth and development of children, and pregnancy complications of low birth weight and increased prenatal mortality. Stunting (low height-for-age) among preschool children is a common clinical manifestation of zinc deficiency (United Nation University 2004). Zinc is also thought to play a role in vitamin A and β -carotene metabolism. Zinc supplements had shown previously to improve dark adaptation and intestinal integrity and zinc deficiency was found to aggravate the clinical effects of vitamin A deficiency (Marjoleine *et al.* 2001).

A poor diet and infection frequently coexist and interact in populations where vitamin A deficiency (VAD) is widespread. In such settings, VAD can increase the severity of infection which in turn, can reduce intake and accelerate body losses of vitamin A to exacerbate deficiency. The prevalence and severity of xerophthalmia, anaemia and the (less-measurable) "vicious cycle" between VAD and infection in vulnerable groups (notably young children and pregnant or lactating mothers) represent the most compelling consequences of VAD and underlie its significance as a public health problem around the world (WHO 2009).

The prevalence of clinical vitamin A deficiency and night blindness among mothers was 1.8% nationally and 1.0% in the southern region of Ethiopia. Nationally, 37.7% of children (95% CI, 35.6% to 39.9%) had deficient serum retinol levels which may be concluded that the prevalence of vitamin A deficiency is significantly higher among children and mothers in the country (Demissie 2010).

Breast-milk vitamin A is a unique indicator for assessing the vitamin A status of lactating mothers and their breast-fed infants, and has recently been recommended by WHO for use in monitoring global elimination of vitamin A deficiency (Stolztus and Underwood 1995). Assessing breast milk vitamin A is less invasive than alternative approaches for assessing a mother's vitamin A status and her infant. Collection of milk samples in the field is generally feasible and acceptable and can serve as an indicator for measuring the impact of vitamin A interventions on women and infants.

The nutritional composition of breast milk in developing countries, especially among malnourished women, has not been fully investigated. The three key factors of maternal nutrition that could have an impact on human milk composition are current dietary intake, nutrient stores, and alterations in nutrient utilization. Alterations in maternal nutrition that change the composition of human milk may have positive, neutral, or negative consequences to the nursing infant (CNSDPL 1991). When malnutrition is sufficiently severe, lactation performance becomes compromised (UNACC 1992). Even though the prevalence of hemoglobin concentration <120 g/L (30.4%) and the prevalence of night blindness (1.8%) among lactating mothers were known (Demissie *et al.* 2010; Haider 2010), the prevalence of zinc deficiency among lactating women is unknown.

Therefore, study on nutritional status of lactating mother is an important health issue since nutritional status may have relationship with the nutrient content of her breast milk. Hence this study was designed to

determine breast milk composition of iron, zinc and vitamin A from women 6 to 12 months postpartum and to correlate with the nutrient adequacy of the lactating mothers using blood biomarkers of iron, zinc and retinol and dietary assessment in a rural area of southern Ethiopia.

Materials and methods

The study area, Boricha district is found in Sidama zone, Southern Nation, Nationalities and People Regional State (SNNP) which is located at 311 km south of Addis Ababa. The district is known to have very high food insecurity level and experiences long periods of increased food deficit due to erratic rainfall pattern and lack of modern agriculture (Aster 2010, personal communication). The total population of the district is 282,310 with 50.1% females and 49.9% males. The estimated rural population is 266,406 among these, 3.5% are lactating mothers. Agriculture is the major source of income with the major crops being maize, haricot bean, sweet potato and *Enset* produced in the district (UNHCO 2005).

A community based cross-sectional survey was conducted from April to May 2012. Lactating mothers who were breast feeding a single child 6-12 months of age, apparently healthy, free from medication, non-pregnant, and gave their written consent were included in this study. Lactating mothers who use hormonal contraceptive were excluded from this study.

In order to get a maximum sample size; 50% population proportion was used and a total of 384 lactating mothers were randomly selected for anthropometric measurement and 24 hour dietary recall. After the first visit of the selected 384 HHs, 120 lactating mothers randomly selected and invited through health posts for plasma, breast milk and repeated 24 hour dietary recall assessment. Except one woman, the others were willing to participate in this study. A maximum of 7ml venous blood sample was collected by a medical laboratory technologist in the morning prior to the first meal of the day.

Hemoglobin analysis was conducted using HemoCue hemoglobin analyzer. For zinc, ferritin, iron and retinol analysis, blood samples were collected using trace element-free test tubes. Within 30 minutes of collection, blood was centrifuged (3500 rpm for 10 minutes) and plasma was transferred to trace element free Nunc tube, covered by aluminium foil to protect from light and transported using dry ice. The samples were stored at -20°C until laboratory analysis. Plasma ferritin was analysed by the Elecsys 2010 immunoassay (Roche diagnostic); plasma retinol was

was determined using HPLC method (EHNRI 2010); and plasma iron and zinc were analyzed using atomic absorption spectroscopy (Perkin Elmer 1996).

Breast milk sample was collected during regular feeding time, two hours after the previous nursing. The breast milk samples were collected in to hard plastic containers with an airtight seal and then delivered on ice to the health centre. The breast milk samples were then stored at -20°C (WHO 2007). Breast milk iron and zinc analyses were done by atomic absorption spectroscopy; retinol by high performance liquid chromatography, protein using Kjeldahl apparatus, and fat analysis done using the *Gerber method*. Breast milk carbohydrate was found by calculating the difference after analyzing the moisture and ash concentration of breast milk.

During the 24-hour recalls, respondents were asked for detailed descriptions of all foods and beverages consumed, including cooking methods and recipes. Every household provided one recall, and from 120 sub-samples, a second recall was collected, for a weekend day. Using a software, nutrient data for each food from food composition table for Ethiopian foods (EHNRI 1997; EHNRI 1998); Tanzanian (Zohra and Ellen 2008) and American food composition tables were combined to provide dietary intakes.

Ethical issues

The study protocol was reviewed and approved by National Scientific and Ethical Review Committee of the Ministry of Science and Technology of Ethiopia. Participants were informed of all study procedures and provided informed written consent.

Results

Maternal nutritional status: Of the 384 lactating mothers, 372 completed anthropometric and dietary data collection (96.9% response rate). For the subset of 120 lactating mothers for biological samples, 109 women (90.8% response rate) had completed the data. Twenty four percent of lactating mothers had BMI below 18.5 kg/m^2 considered to be underweight, while only 1.5% had BMI above 25 kg/m^2 considered being overweight.

The prevalence of anemia among lactating mothers aged 15-49 years was 36%, with 18.4% having mild anemia, 17.5% moderate anemia, and 0.8% severe anemia. In addition, 10.6 % of lactating mothers had ferritin levels below $15 \mu\text{g/L}$. The prevalence of zinc and vitamin A deficiencies were 100 % and 7.3%, respectively (Table 1).

Table 1: Anthropometry and blood analysis of the lactating mothers by months postpartum, in Boricha District, Sidama Zone, Ethiopia, 2012

Characteristics	6-8 months (n = 187)	9-12 months (n = 185)	Total (n = 372)
Anthropometry			
Height (cm) ^a	155.45 $\pm$ 6.2	156.16 $\pm$ 5.7	155.80 $\pm$ 6.0
Weight (kg) ^a	48.49 $\pm$ 6.3	48.74 $\pm$ 6.3	48.61 $\pm$ 6.3
BMI (kg/m^2) ^a	20.0 $\pm$ 2.1	20.0 $\pm$ 2.4	20.0 $\pm$ 2.2
% (BMI < 18.5) ^b	12.04 (45)	12.04 (45)	24.07 (90)
% (BMI 18.5-24.9) ^b	37.35 (139)	37.04 (138)	74.38 (277)
% (BMI $\geq$ 25) ^b	0.93 (3)	0.62 (2)	1.54 (5)
MUAC (cm) ^a	23.9 $\pm$ 2.2	23.8 $\pm$ 2.7	23.8 $\pm$ 2.5
% MUAC (< 21cm) ^b	3.5 (13)	1.9 (7)	5.4 (20)
% MUAC ($\geq$ 21cm) ^b	47.2 (175)	47.5 (177)	94.6 (352)
Blood analysis			
Hb (g/L) ^a	130.5 $\pm$ 26	130.6 $\pm$ 22	13.05 $\pm$ 24
% (Hb < 120 g/L) ^b	12.5 (15)	16.7 (20)	29.2 (35)
% Mild (100 - 119g/L) ^b	6.7 (8)	11.7 (14)	18.4 (22)
% Moderate (70-99 g/L) ^b	5.8 (7)	11.7 (14)	17.5 (21)
% Severe (Hb < 70 g/L) ^b	0.8 (1)	0.0 (0)	0.8 (1)
Plasma Ferritin ($\mu\text{g/L}$) ^a	42.25 $\pm$ 32.0	45.92 $\pm$ 43.8	44.37 $\pm$ 39.1
% (PF < 15 $\mu\text{g/L}$) ^b	3.2 (4)	7.4 (9)	10.6 (13)
Plasma iron ($\mu\text{g/L}$) ^c	1.84 (1.41, 2.24)	1.77 (1.42, 2.05)	1.78 (1.42, 2.16)
Plasma Zinc (mg/L) ^a	0.11 $\pm$ 0.03	0.10 $\pm$ 0.03	0.10 $\pm$ 0.03
%(PZn < 0.7 mg/L) ^b	41.1(45)	58.9(64)	100 (109)
Plasma Retinol ($\mu\text{g/dL}$) ^a	58.6 $\pm$ 20.8	60.1 $\pm$ 33.2	59.5 $\pm$ 28.4
% (PR $\leq$ 30 $\mu\text{g/dL}$) ^b	4.2 (5)	3.1(4)	7.3 (9)

^aMean $\pm$ SD, ^bpercent (Number), ^cMedian (1st, 3rd quartile), BMI- Body Mass Index; Hb- Hemoglobin; PF- Plasma Ferritin; PR- Plasma Retinol; PZn- Plasma Zinc. BMI < 18.5 is under weight, BMI 18.5-24.9 is normal weight, BMI > 25 is overweight.

The median energy intake of lactating mothers was 1560 kcal/d, corresponding to 33.7 % of Estimated Average Requirement (EAR). The protein intake was 31.6 g/d. The vitamin C intake was very low at 24.8% of EAR. The median iron (assuming 5%

bioavailability), zinc (assuming low bioavailability) and vitamin A intake of the lactating mothers were 1.44 mg/d, 0.95 mg/d and 113.32µg/d respectively (Table 2).

Table 2: Energy and nutrient intakes of lactating mothers in Boricha district, Sidama Zone, Ethiopia, 2012

Indicators	Median (1st , 3rd quartiles) intake per day	EAR
Energy (kcal/d)	1560 (1150, 1950)	1757
Protein (g/d)	31.6 (18.5, 47.0)	39.2
Iron (mg/d)	1.44 (1.01, 1.94)	23.6
Zinc (mg/d)	0.95 (0.67, 1.31)	4.8
Vitamin A (RE µg/d)	113 (49.6, 472)	450
Calcium (mg/d)	880 (621, 1246)	833
Vitamin C (mg/d)	1.4 (0.1, 57.9)	58

Breast milk composition: The nutrient composition of breast milk samples (n=115) is shown in Table 3. The mean energy concentration was lower (574.4± 75.1).

Breast milk iron and zinc contents were very low, 0.24 ± 0.08 and 0.08 ± 0.06 mg per liter, respectively.

Table 3: Composition of Human Milk Collected from Lactating Mothers 6-12 months Postpartum in Boricha District, Sidama Zone, Ethiopia, 2012

Indicators	6-8 months (n = 47)	9- 12 months (n = 68)	Total (n = 115)	Other findings 6-12 months Postpartum
Energy (Kcal/L)	573.58 ± 77.8	575.05 ± 74.3	574.40 ± 75.1	642.90 ± 26.2 ^a
Fat (g/L)	22.86 ± 8.3	24.32 ± 8.9	23.71 ± 8.6	39.60 ± 2.1 ^a
Protein (g/L)	9.45 ± 1.7	9.63 ± 2.1	9.55 ± 1.9	8.20 ± 0.5 ^a
Carbohydrate (g/L)	84.05 ± 6.5	84.18 ± 8.5	84.12 ± 7.7	-
Iron (mg/L)	0.24 ± 0.1	0.24 ± 0.1	0.24 ± 0.1	0.43 ± 0.15 ^b
Zinc (mg/L)	0.08 ± 0.03	0.09 ± 0.07	0.08 ± 0.06	0.56(0.37,0.82) ^b
Vitamin A (µg/dL)	12.88 ± 0.02	12.85 ± 2.1	12.86 ± 2.2	-

For Fat analysis 60 samples were used for analysis because of lack of enough amount of sample for those analyses.

^a Breast milk composition results studied by Mitoulas *et al.*(2002).

^b Breast milk iron level of Vietnamese women who had iron supplement reported by Nakamori *et al.* (2009).

Correlation between maternal nutritional status and breast milk content: Correlations between dietary intake of lactating mothers and their breast milk concentrations of iron, zinc and vitamin A are shown in Table 4. There were no significant correlation for iron and vitamin A. However, there was a significant inverse correlation for zinc ($r = -0.184$; $p < 0.05$).

Table 4: Correlation (r) between breast milk trace element concentrations and dietary nutrient intake/plasma levels of lactating women in Boricha district, Sidama Zone, Ethiopia, 2012

Breast milk (N = 97)	Dietary	r	P value
Iron	Iron	-0.102	0.28
	Protein	0.029	0.76
	Energy	-0.020	0.84
Zinc	Zinc	-0.184	0.05
	Protein	-0.121	0.20
	Energy	-0.156	0.10
Vitamin A (retinol)	Vitamin A (β-carotene)	-0.140	0.14
	Protein	0.018	0.85
	Energy	-0.008	0.94
Breast milk (N = 96)	Plasma	r	P value
Iron	Ferritin	-0.014	0.89
	Iron	0.088	0.42
	Hemoglobin	-0.31	0.75
Zinc	Zinc	-0.08	0.45
Vitamin A	Vitamin A (retinol)	0.227	0.03

There was a significant positive correlation between the concentration of retinol in breast milk and maternal plasma ($r=0.227$; $p<0.03$). However, there was no correlation between ferritin, iron and zinc concentrations in breast milk with the corresponding plasma level (Table 4).

Correlation between maternal plasma level of trace elements and dietary intake: There was a significant positive correlation between plasma retinol and the dietary intake of vitamin A (β -carotene) ($r=0.223$; $p<0.03$). However, plasma zinc concentration showed inverse correlations with dietary intake of protein and of energy (Table 5).

Table 5: Correlation (r) between plasma trace element concentrations and dietary intake of lactating women in Boricha district, Sidama zone, Ethiopia, 2012

Plasma trace elements (N = 97)	Dietary intake	r	P value
Iron	Iron	-0.068	0.53
	Protein	0.000	0.99
	Energy	-0.083	0.44
Zinc	Zinc	-0.166	0.11
	Protein	-0.205	0.05
	Energy	-0.205	0.05
Vitamin A (retinol)	Vitamin A (β -carotene)	0.223	0.03
	Protein	-0.053	0.60
	Energy	0.190	0.06

Discussion

This study is the first to measure the concentration of iron, zinc and vitamin A in human milk in lactating rural Ethiopian women, along with assessing dietary intake, anthropometric status and plasma nutrient levels.

The study population showed a mean height (155.8 ± 6.0 cm) similar to the national average (Abuye *et al.* 2010) and previous survey finding in the same region (Abebe and Bogale 2008). Mean weight in this study was 48.61 ± 6.0 kg showing high prevalence of chronic energy deficiency, with approximately one-quarter being classified as underweight (BMI <18.5). This value is higher than that of a survey data for the Southern Regional State (20 %) (EDHS 2012), but comparable to values reported for a study in the same region (Rosalind *et al.* 2008). Both short stature and low body mass index are significant risk factors for low birth weight deliveries (Deshmukh *et al.* 1998).

The result of this study showed that the median intake of iron (1.44 mg per day), zinc (0.95 mg per day) and vitamin A (retinol, 113. μ g per day) of lactating women were very low compared to studies reported for other developing countries (Masay *et al.* 2009; Kawatra and Salil 1998). This might be due to the relatively high proportions of energy provided by *Enset* products in southern Ethiopia which contains very little zinc, iron and vitamin A, and lower consumption of animal source of foods. *Enset* products such as *kocho*, consisting of the fermented pulp of *enset* baked into bread were most frequently

eaten, followed by *bulla* (white low-fiber desiccated juice from *enset* pulp) and unfermented maize bread. Analysis of samples of fermented *kocho* and *bulla* showed zinc content (per 100 g fresh weight) of 0.52 mg and 0.07 mg, respectively; of iron level of 6.2 mg and 4.8 mg, respectively with low bioavailability (Abebe *et al.* 2007).

Vitamin A-RE content of fermented *kocho* and *bulla* consumed were also very low (19.4 and 1.3 RE μ g/100g fresh weight respectively). Unfermented maize bread only contains iron (2 mg.), zinc (1.24 mg) and vitamin A (β -carotene, 0 RE μ g) per100g of the food, respectively (EHNRI 1998). Plasma retinol concentration of lactating mothers in Boricha district indicated that 7.3% had plasma vitamin A level below 30 μ g/dL (0.7 μ mol/L) indicating vitamin A deficiency, a level higher than a recent WHO report on Ethiopia showing a prevalence of 4.2% (WHO 2009).

Anemia was found in 36% of the lactating mothers studied, again higher than that found (25%) among women of reproductive age in southern Ethiopia (Umata *et al.* 2008). The prevalence of zinc deficiency was 100% of lactating mothers studied, higher than the other study results (76%) conducted on pregnant women of Southern Ethiopia (Stocker *et al.* 2009). The current study population lived in a food insecure district dependent on starchy foods; therefore the higher prevalence of zinc deficiency in the study area is not unexpected. These might be due to the lower value of human milk fat concentration (23.71 ± 8.6) in this study as compared to the finding of Mitoulas *et al.* (2002).

Breast milk concentrations are affected by the time of day and the time of sampling during feeding (Masay *et al.* 2009). Breast milk was collected in the morning; between 8 and 9 am more than an hour after the previous breastfeeding. Variation of breast milk zinc concentration in the study participant showed relatively lower standard deviation (0.06 mg/L) and CV (75%) compared to other studies regulated by the timing of collecting breast milk (Shahnaz 2010; Heloisa *et al.* 2002). The concentration of zinc, iron, fat and energy were relatively lower than other comparable studies conducted in developing countries (Masay *et al.* 2009; Mitoulas *et al.*, 2002).

The concentration of iron, zinc and retinol in breast milk is considered low in relation to plasma iron, zinc and retinol concentration. Breast milk iron concentration was 14% of plasma iron, breast milk zinc was 80% of plasma zinc and breast milk retinol was 22 % of plasma retinol concentration. In this study, breast milk concentration of zinc is not correlated with either dietary intake or plasma zinc concentration. This result is similar to the study conducted on Vietnamese lactating mothers where their zinc store and intake was higher (Masay *et al.* 2009).

Although anemia was highly prevalent among lactating mothers, it was not correlated with breast milk iron concentration. This result was comparable with the study results of Shashirja *et al.* (2006). Breast milk iron concentration appears to be constant whether women have low blood levels, as found in this study which is in agreement with values from a study done on Nigerian lactating women supplemented with iron (Arnaud *et al.* 1993).

In this study, there was no correlation of maternal dietary intake of vitamin A (primarily as β -carotene) and breast milk concentration of retinol. In contrast, a study reported by Grazyna *et al.* (2009) stated that dietary vitamin A (β -carotene) intake was correlated with the concentration of retinol in breast milk although vitamin A status and their intake had differences with this study population. The present study results showed that breast milk vitamin A concentration was positively correlated with plasma retinol in lactating mothers in agreement with study results of Grazyna *et al.* (2009).

Conclusion

In this study, the prevalence of iron deficiency anemia, zinc and vitamin A deficiency among lactating mothers, 6 to 12 months postpartum were high: 36%, 100% and 7.3%, respectively. The median calorie and intakes of iron, zinc and vitamin A were inadequate. The average energy, fat, iron and zinc content of breast

milk were lower than other comparable studies done in other developing countries. The breast milk content of iron and retinol is not correlated with dietary intakes; however, breast milk zinc showed a negative correlation with dietary intake of zinc. Plasma concentration of retinol showed a significant correlation with that of breast milk retinol concentration, but plasma concentrations of iron and zinc showed no correlation with breast milk levels.

Competing interests

The authors declare that they have no competing interests.

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