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Research Article
Mineral Concentrations in Breast Milk across Infant Birth Weight

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Abstract
Objectives: This study was conducted to assess the mineral concentrations of calcium, zinc and iron in breast milk based on infant birth weight. **Materials and Methods:** This was a descriptive cross sectional study that used random sampling. The sample included 37 nursing mothers, consisting of 31 mothers with normal weight babies and six mothers with low birth weight (LBW) babies. Data were collected using a questionnaire and 30 mL breast milk samples were analyzed using anatomical absorption spectrophotometry (AAS). **Results:** There were differences in micronutrient levels between LBW and normal weight infants. Calcium and zinc levels were higher in LBW infants, while iron content tended to be higher in normal weight infants. **Conclusion:** Despite some differences, the micronutrient content was similar in the breast milk of mothers who gave birth to LBW and normal weight infants.

Key words: Breast milk, infant, low birth weight, micronutrients, nutrition

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast milk contains macronutrient and micronutrient content produced according to the needs of infants. It also contains a variety of bioactive molecules that protect infants against infection and inflammation, as well as contribute to maturation of organs and healthy microbial colonization[1].

The World Health Organization recommends that infants be breastfed as soon as possible after birth; this includes infants born with a low birth weight (LBW)[2]. Studies that examined levels of iron, copper, zinc, calcium and magnesium in breast milk have found that all micronutrients, except for calcium, are produced based on the infants' nutritional needs[3].

The micronutrient content of breastmilk is affected by several factors, such as stage of lactation and micronutrient levels in the mother's blood serum, as well as other maternal factors like dietary supplementation[4]. Studies have shown that micronutrient content in breast milk decreases significantly from week 2 to month 7 post-partum[5]. One study showed that maternal serum micronutrient content is not related to micronutrient content.

Maternal iron status is correlated with iron content in breast milk[6]. One study showed that supplements and other food consumed by the mother did not affect micronutrient levels[7]. The micronutrient content of breast milk may not be affected by nutritional and socioeconomic status, diet, supplementation, age, number of births, or use of contraceptives, although environmental factors, such as the mother's residence, may affect the content of breast milk[4].

A study on nursing mothers from urban and sub-urban areas found significant differences in breast milk between the groups based on where they lived[8]. A meta-analysis of studies on micronutrient levels in term and pre term infants showed no differences in breast milkcontent[9]. However, one study showed that the iron content in breast milk from mothers who gave birth to premature babies was higher than mothers who gave birth to normal weight babies[10].

LBW infants weigh less than 2,500 g at birth. While various formulas have been created for LBW infants, breastfeeding alone may meet their needs. This study aimed to determine if breast milk contains sufficient micronutrient content, particularly calcium, iron and zinc, in normal weight and LBW infants.

MATERIALS AND METHODS

This study was conducted at the Kassi Kassi Health Center in Makassar, Indonesia from May through June 2016. The study design is cross sectional.

Participants: Mothers and children registered at the Kassi-Kassi Health Center were included in this study. Breast-feeding mothers who met the inclusion criteria and were willing to participate in the study provided informed consent. Random sampling was used. The criteria for inclusion included birth to a child who was breastfed; mothers who gave birth to full-term (up to 6-week-old infants) were also included. The minimum sample size was calculated based on 37 nursing mothers.

Data collection: Data collected included maternal nutrition and infant birth weight; breast milk samples were collected and analyzed. Nutrient intake data were collected by 24 h food recall questionnaires to assess eating habits. Data were processed using a nutrisurvey. Maternal intake data were then compared to Recommended Dietary Allowances (RDA) guidelines. LBW was defined as <2,500 g and $\geq$2,500 g was considered normal. Milk samples were collected throughout various steps.

The first step included squeezing the milk. Mothers sat leaning forward to stimulate the flow of breast milk. Funnel pumps were mounted in the nipple. The second stage was pumping, which is done slowly and steadily. An electric breast pump can pump milk in15 min. If the milk flow stopped during pumping, the breast was massaged and rested. The third stage is breast milk storage. Glass bottles or plastic containers with tight lids were used to store the milk. Each container was labeled with a code and the name of the mother. Samples were subsequently confirmed as tightly closed and stored in a freezer. The ASI AAS method was used to analyze mineral levels in breast milk. The tools used included Tanur, Pumpkin Measure 50 mL, a set of tools AAS and analytical scales.

Data analysis: Data were analyzed using SPSS. Univariate analysis was conducted to see if the distribution of frequencies and percentages of each variable explained the characteristics of each variable. The data were presented as mean$\pm$standard deviation (SD).

RESULTS AND DISCUSSION

There were more female LBW infants (18.8%) compared to males (14.3%) (Table 1). Mothers aged 20-35 years old (17.9%), an age group that dominated the sample, were more likely to give birth to LBW infants. Mothers who gave birth to LBW infants had basic education (18.2%) and had chronic energy malnutrition during pregnancy (28.6%). Most LBW infants were 2-3 weeks old at the time of the study (20.0%).

Table 1: Characteristics of respondents

	Socio-economic status and birth weight					
	Normal (n = 31)		Low (n = 6)		Total	
Characteristics	No.	Percentage	No.	Percentage	n = 37	Percentage
Gender of infants						
Male	18	85.7	3	14.3	21	56.8
Female	13	81.3	3	18.8	16	43.2
Age group of mothers						
<16 years	1	100.0	0	0.0	1	2.7
16-19 years	2	100.0	0	0.0	2	5.4
20-35 years	23	82.1	5	17.9	28	75.7
35-45 years	5	83.3	1	16.7	6	16.2
Age group of infants						
2-3 weeks	12	80.0	3	20.0	15	40.5
4-6 weeks	19	86.4	3	13.6	22	59.5
Education of mothers						
Primary education	9	81.8	2	18.2	11	29.7
Higher education	22	84.6	4	15.4	26	70.3
Status upper arm circumference pregnancy						
Non chronic energy malnutrition	26	86.7	4	13.3	30	81.1
Chronic energy malnutrition	5	71.4	2	28.6	7	18.9

Table 2: Comparison of mothers' nutrient intake

	Nutritional status and birth weight			
	Normal (n = 31)	Low (n = 6)	Total	p-value
Nutrients	Mean±SD	Mean±SD	Mean±SD	
Intake				
Energy kcal^{-1}	1,799.76±514.11	1,776.38±436.89	1,795.97±496.84	0.918
Protein g^{-1}	66.39±23.38	62.01±19.49	65.68±22.60	0.670
Fats g^{-1}	59.06±30.12	57.28±27.63	58.77±29.37	0.894
Carbohydrate g^{-1}	252.14±67.40	254.78±69.73	252.57±66.80	0.931
Zinc mg^{-1}	6.45±2.22	5.70±2.33	6.33±2.22	0.454
Calcium mg^{-1}	384.17±311.66	280.92±218.47	367.43±298.43	0.446
Iron mg^{-1}	8.60±5.23	7.26±3.80	8.39±5.00	0.554
Intake percent by RDA 2013				
Energy kcal^{-1} (%)	71.68±20.87	69.35±17.14	71.30±20.11	0.799
Protein g^{-1} (%)	86.25±30.72	81.48±25.75	85.48±29.69	0.724
Fats g^{-1} (%)	77.35±41.01	68.75±31.79	75.95±39.40	0.631
Carbohydrate g^{-1} (%)	70.09±18.43	71.42±19.17	70.31±18.29	0.874
Zinc mg^{-1} (%)	42.32±15.30	38.00±15.65	41.62±15.23	0.533
Calcium mg^{-1} (%)	30.99±26.08	21.88±16.78	29.52±24.85	0.419
Iron mg^{-1} (%)	26.95±16.32	22.72±11.90	26.26±15.62	0.551

Table 2 shows that maternal intake during pregnancy differed between mothers who gave birth to normal weight and LBW infants, although the difference was not significant. Pregnant women who gave birth to a normal weight infant had higher intake of energy, protein, fats, zinc, calcium and iron than pregnant women who gave birth to LBW infants; however, carbohydrate intake was higher in pregnant women who gave birth LBW infants. Intake according to RDA guidelines demonstrated similar findings.

Micronutrient (calcium, zinc and iron) levels did not differ between normal weight and LBW infants; however, there were numerically higher mean levels of zinc in LBW (1.13+0.67 mg L^{-1}) compared to normal birth weight infants (0.84+0.52 mg L^{-1}). Mean calcium levels were also numerically higher in LBW infants (381.73+115.80 mg L^{-1}) compared to normal birth weight infants (336.99+75.20 mg L^{-1}). Numerically mean iron levels were found higher in normal birth weight infants (5.24+9.10 mg L^{-1}) compared to LBW infants (2.06+1.53 mg L^{-1}).

Table 3: Comparison of mineral concentration of breast milk

Mineral concentration of breast milk (units L^{-1})	Weight Normal (n = 31) Mean±SD	Weight LBW (n = 6) Mean±SD	Total Mean±SD	p-value
Zinc mg^{-1}	0.84±0.52	1.13±0.67	0.88±0.55	0.230
Calcium mg^{-1}	336.99±75.20	381.73±115.80	344.25±82.79	0.231
Iron mg^{-1}	5.24±9.10	2.06±1.53	4.73±8.41	0.404

Breast milk is the best food for infants; however, in certain circumstances, it may not be sufficient. LBW infants or those born less than 35 weeks old may need higher protein and energy levels to support their growth[11].

LBW in infants is a public health problem, as it is associated with increased infant morbidity and mortality. Approximately 95% of infants with LBW are born in developing countries. Birth weight is influenced by genetic, reproductive, obstetric, social and environmental factors. However, LBW in infants is also related to early marriage, the mother's nutritional status and anemia and other infections. LBW is usually preceded by malnutrition in women who are shorter and have smaller infants[12].

This study found that infants with LBW were more likely to be female. A study conducted by Thomson *et al.*[13] in pregnant women in Abardee between 1948-1964 found no gender differences in birth weight at up to 34-35 weeks of gestation. After 38 weeks of gestation, however, males weighed 150 g or more[13]. Another study showed differences in weight based on sex starting in the first trimester of pregnancy[14].

This study found that LBW incidence was more common in women age 20-35 years, those with primary school education and those who suffered from chronic energy malnutrition during pregnancy. One study found that the incidence of LBW significantly occurs in women younger than 19 years old and in women over the age of 40. For very young mothers, the incidence of LBW may be related to maternal readiness and poor nutritional status, as their reproductive organs are immature. For older mothers, the incidence of LBW may be associated with increased spacing between births[12]. Another study found that mothers younger than 21 years had a low risk of having a LBW infants; however, mothers over 35 years had a higher risk[15]. In this study, LBW incidence was more common in women 20-35 years; this is likely due to sample bias. Educational status was also related to LBW. Based on the incidence of malnutrition during pregnancy, prenatal care and weight gain during pregnancy were insufficient. All of these factors are significant predictors of LBW incidence[16].

This study found that zinc and calcium levels were higher in LBW infants compared to normal birth weight infants, while iron levels were higher in normal birth weight infants. An analysis of nutrient content in the milk of preterm and full-term babies found that levels of minerals, including calcium and phosphate, were mostly similar between premature and full-term infants[9]. The findings are similar to our results.

Micronutrient content of breast milk is influenced by lactation stage and micronutrient content declines over time. The highest micronutrients are found in the colostrum. A study found that zinc in the colostrum is 5.32 mg mL^{-1} and decreased to 1.12 mg mL^{-1} after seventh months[17]. Iron content in the colostrum was found to be 0.56 mg L^{-1} and decreased to 0.39 mg L^{-1} on postpartum day 30[5]. Febria *et al.*[18] observed that calcium levels in breast milk of infants aged 6-12 months decreased to 125.4 mg L^{-1}. In infants aged 2-6 weeks, calcium content is 336.99 mg L^{-1} in normal birth weight infants and 381.73 mg L^{-1} in LBW infants.

Zinc is an essential micronutrient that acts as a structural, catalytic, or regulator for over 300 enzymes and degrades carbohydrates, lipids, proteins and nucleic acids. Zinc plays a key role in the synthesis and stabilization of genetic material and is required for cleavage[19]. A study assessing the nutritional status and nutrient content in the milk of mothers in Vietnam found that breastfeeding mothers who suffer from anemia have low serum zinc concentrations, although no relationship was found between iron, zinc and copper and micronutrient intake[20]. One study found no differences in the zinc content of the milk of mothers between those with good nutritional status and those who suffered from chronic energy loss postpartum[21]. Another study found that zinc supplementation did not have a significant effect on zinc content in breast milk[22].

Hemoglobin, red blood cell and iron profiles are significantly different in infants born to mothers with anemia, as micronutrient content of breast milk is significantly decreased in women with anemia. Anemia in pregnant women also affects iron levels in the placenta and breast milk[23]. Shahiraj *et al.*[24] found that iron content in breastmilk decreases rapidly from day 1 through week 14 and at month 6; there were no significant differences compared to women with anemia. Significant reductions were seen in levels of lactoferrin in breast milk from day 1 to week 14 in women with anemia. Hemoglobin and serum ferritin were not related to iron or lactoferrin levels either on day 1, week 14, or 6 months postpartum[24].

CONCLUSION

Micronutrient (calcium, zinc and iron) content was similar in the breast milk of mothers who gave birth to LBW and normal weight infants.

SIGNIFICANCE STATEMENT

This study found that micronutrient levels, including calcium, zinc and iron, were similar in breast milk from mothers who gave birth to normal weight or LBW infants. This study will help researchers better understand micronutrient levels in breast milk.

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