

## Zinc Concentrations in Breast Milk Samples Obtained from Palestinian Lactating Women: A Cross-Sectional Study of Signs of Deficiency and Dietary Consumption of Zinc Sources

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**Abstract:** Zinc is one of the most crucial trace minerals that is needed for human health and development. This study was conducted to assess zinc concentrations in breast milk samples obtained from Palestinian lactating women. The study also aimed to assess associations between breast milk zinc concentrations, dietary zinc intake, and signs and symptoms of zinc deficiency. Breast milk samples were obtained from 58 lactating women. The demographic variables, dietary zinc intake, and signs and symptoms of zinc deficiency were also collected. Zinc concentrations were assessed using a graphite furnace atomic absorption spectrophotometric method. Of the lactating women, 7 (12.1%) reported loss of appetite, 6 (10.3%) reported sleepiness or lethargy, 11 (19.0%) reported changes in weight, 7 (12.1%) reported increasing hair loss, 8 (13.8%) reported having easily broken nails, 7 (12.1%) reported having immune diseases/issues, and 11 (19.0%) reported dermatologic issues. In this study, 21 (36.2%) of the lactating women reported high consumption of red meats or poultry, 22 (37.9%) reported high consumption of milk or dairy products, 19 (32.8%) reported high consumption of whole grains, 18 (31.0%) reported high consumption of fish or seafood, 12 (20.7%) reported high consumption of eggs, and 9 (15.5%) reported high consumption of zinc-rich drinks. In this study, the median breast milk zinc level was 0.091 [0.063, 0.15] mg/100 mL. Breast milk zinc concentrations were higher among the lactating women who were younger than 30 years, lived in urban areas, were employed, and reported high consumption of zinc-rich drinks. Breast milk zinc concentrations were affected by some demographic variables of the lactating women. These variables included, demographics, living conditions, and consumption of zinc-rich sources. Dieticians, lactation consultants, and other healthcare providers should educate/counsel lactating women on the importance of maintaining adequate breast milk zinc levels and consuming zinc-rich sources. More studies are still needed to assess the impact of zinc supplements on breast milk zinc concentrations.

**Keywords:** Zinc, Breast Milk, Breastfeeding, Infants, Women Health.

### Introduction

Breast milk is the most suitable source of micro- and macronutrients for infants in the first 6 months of their lives [1]. Breast milk contains essential micro- and macronutrients for the normal maturation and development of infants [1-3]. In the absence of contraindications, health authorities and professional bodies recommend that all infants should be breastfed exclusively for the first 6 months of their lives [4]. After that, breastfeeding can be continued along with complementary foods until the age of 2 years or beyond.

It is important to note that human milk is composed of a wide range of chemical and biological elements [1]. Previous research has found qualitative and quantitative variations in micronutrients, proteins, fats, carbohydrates, hormones, electrolytes, vitamins, and microbial contents in breast milk samples [1-3]. Inadequate amounts of essential constituents in breast milk may harm the normal maturation and development of infants.

Zinc is one of the most crucial trace minerals that is needed for human health and development [5]. Zinc deficiency was common among lactating women [6]. Therefore, zinc deficiency has historically been recognized as a public health issue. Zinc deficiency can be associated with immunological deficiencies,

delayed wound healing, diarrhea, growth failure, and neurodevelopmental problems among infants [5, 7]. Moreover, zinc deficiency can increase the risk of pneumonia and diarrhea. On an annual basis, zinc deficiency could be responsible for more than 450,000 child deaths [8].

Because of the serious deleterious consequences of zinc deficiency, different European nutrition societies recommended lactating women to take zinc supplements [9]. The dose of the recommended zinc supplement varies depending on the intake of dietary phytates that reduce the absorption of zinc [9]. Therefore, assessing breast milk zinc concentrations could be an essential biomonitoring step in combating zinc deficiency among breastfeeding women and their infants [10, 11]. In a systematic review and meta-analysis of previous studies, the mean breast milk zinc concentration was 2.57 mg/L (95% CI: 2.50, 2.65) [12]. The studies included in this systematic review and meta-analysis originated from countries in Europe, North and South America, Asia, Africa, and Australasia/Oceania. Breast milk zinc concentrations were affected by maternal age and dietary habits. Little studies were conducted in Palestine and other Arab countries [2, 13]. Moreover, little studies assessed breast milk zinc concentrations as associated with the maternal demographic variables and dietary intake habits. In addition,

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relationships between breast milk zinc concentrations with signs and symptoms of zinc deficiency were not assessed before. Therefore, this study was done to assess breast milk zinc concentrations among lactating Palestinian women. The study also aimed to assess associations between breast milk zinc concentrations, dietary zinc intake, and signs and symptoms of zinc deficiency.

## Methods

**Study design:** A cross-sectional observational study was conducted in the northern regions of the West Bank. Due to the possibility of meeting and inviting lactating women to participate in the study, maternity care centers (n = 4) in the north of the West Bank were used as the sampling frame. The lactating women were asked to provide breast milk samples in the period between November 2022 and May 2023.

**Sample size and sampling:** The sample size was calculated as informed by previous studies that assessed breast milk zinc concentrations [2, 14]. The following equation was used to compute the sample size for this study. The sample was computed at a 95% confidence interval (95% CI):

$$n = \frac{Z^2 \sigma^2}{D^2}$$

In this equation, n was the sample size, Z at 95% CI was 1.96,  $\sigma$  was the standard deviation (SD) of zinc in breast milk, and D was the precision. In previous studies, the SD of breast milk zinc was in the range of 0.09 to 3.3 [2, 15]. Using a precision (D) of 0.02, the sample size needed was 77 breast milk samples.

In this study, breast milk samples were obtained from women who were 18 years or older, had a term normal vaginal delivery, were lactating for more than 15 days, had healthy babies, and provided written informed consent. Inclusion of women who were lactating for more than 15 days should have avoided the collection of colostrum. It is well-established that colostrum contains high proteins and low in fats compared to mature milk. Women who had chronic diseases and those whose babies had metabolic disorders were excluded.

Breast milk samples (about 5 mL) were collected by the women themselves in mineral-free tubes (Vacutest Kima, Arzergrande, Italy) using a hand expression approach [16]. Before breast milk collection, the lactating women cleaned their chest areas with alcohol wipes. Foremilk samples were collected during morning visits (in the time window between 9:00 am to 11:30 am). Collection of the breast milk samples within this time window should have reduced variabilities in contents and flow rate. Samples were transported to the analytical laboratory at 4 °C and were then frozen at -20 °C until the time of analysis.

In addition to breast milk samples, the women were asked to answer items in a paper-based questionnaire. The paper-based questionnaire gathered the sociodemographic variables of the women like employment status, educational level, place of residence, age, lactation period, and gender of the child. The women were also asked to report signs and symptoms of zinc deficiency, and consumption patterns of zinc sources [2, 13, 15].

**Analytical procedure:** A modifier solution of 0.2 g ammonium dihydrogen phosphate, 0.2 mL nitric acid (concentrated), and 0.5 g Triton X-100 was prepared. The mixture was then diluted in double distilled water to a volume of 100 mL [13, 16, 17]. Nitric acid (10%) was used

to wash all glassware and tubes to prevent the adsorption of zinc on the surfaces. The chemical used in this study were of atomic absorption spectrometric quality. Each sample (0.2 mL) was mixed with 0.8 mL of the matrix solution and then the mixture was sonicated for 5 min before being transferred into the analysis cell. Zinc concentrations were assessed using a graphite furnace atomic absorption spectrophotometric method (iCE™ 3500 Atomic Absorption Spectrometer; Thermo Scientific) [13, 16, 17]. This technique is one of the most commonly used techniques to assess the concentrations of metals and trace elements in biological samples including blood, saliva, and breast milk [13, 16, 18]. Each milk sample was analyzed separately. A similar matrix was used to build the calibration curve. The calibration curve was judged as excellent as indicated by an R<sup>2</sup> of 0.99.

**Data analysis:** IBM SPSS v.21.0 was used to analyze the data. Because of the small sample size and nature of the data, Mann-Whitney U tests were used to compare breast milk zinc concentrations between the groups. Statistical significance was indicated by a p-value of < 0.05.

**Ethical approval:** The Institutional Review Board (IRB) of An-Najah National University provided ethical approval to conduct this study. The IRB approval number given for this study was IRB: #Nsg. Oct. 2023/30. All women who donated breast milk samples gave written informed consent.

## Results

**Demographic characteristics of the women:** In this study, 58 lactating women provided breast milk samples. Of the lactating women, 30 (51.7%) were younger than 30 years, 31 (53.4%) lived in urban areas, 39 (67.2%) had a university education, 47 (81.0%) were unemployed, and 39 (67.2%) were lactating for less than 6 months. The detailed demographic variables of the lactating women are provided in Table 1.

**Table (1):** Demographic characteristics of the lactating women.

Variable	n	%
Maternal age (years)		
< 30	30	51.7
≥ 30	28	48.3
Place of residence		
Urban	31	53.4
Rural	27	46.6
Educational level		
School	19	32.8
University	39	67.2
Employment status		
Unemployed	47	81.0
Employed	11	19.0
Lactation period (months)		
< 6	39	67.2
≥ 6	19	32.8
Gender of the child		
Male	22	37.9
Female	36	62.1

**Signs and symptoms of zinc deficiency:** Of the lactating women, 7 (12.1%) reported loss of appetite, 6 (10.3%) reported sleepiness or lethargy, 11 (19.0%) reported changes in weight,

7 (12.1%) reported increasing hair loss, 8 (13.8%) reported having easily broken nails, 7 (12.1%) reported immune diseases/issues, and 11 (19.0%) reported dermatologic issues. The prevalence of reported signs and symptoms of zinc deficiency is shown in Table 2.

**Table (2):** Prevalence of signs and symptoms of zinc deficiency.

Sign	n	%
<b>Loss of appetite</b>		
No	51	87.9
Yes	7	12.1
<b>Sleepiness/lethargy</b>		
No	52	89.7
Yes	6	10.3
<b>Changes in weight</b>		
No	47	81.0
Yes	11	19.0
<b>Hair loss</b>		
No	51	87.9
Yes	7	12.1
<b>Easily broken nails</b>		
No	50	86.2
Yes	8	13.8
<b>Immune diseases/issues</b>		
No	51	87.9
Yes	7	12.1
<b>Dermatologic issues (dryness/scaling)</b>		
No	47	81.0
Yes	11	19.0

**Consumption of zinc-rich sources:** In this study, 21 (36.2%) of the lactating women reported high consumption of red meats or poultry, 22 (37.9%) reported high consumption of milk or dairy products, 19 (32.8%) reported high consumption of whole grains, 18 (31.0%) reported high consumption of fish or

seafood, 12 (20.7%) reported high consumption of eggs, and 9 (15.5%) reported high consumption of zinc-rich drinks. The reported consumption patterns of zinc sources are shown in Table 3.

**Table (3):** Consumption patterns of zinc sources.

Consumption pattern	n	%
<b>Red meat/poultry</b>		
Low/moderate	37	63.8
High	21	36.2
<b>Milk and dairy products</b>		
Low/moderate	36	62.1
High	22	37.9
<b>Whole grains</b>		
Low/moderate	39	67.2
High	19	32.8
<b>Fish and seafood</b>		
Low/moderate	40	69.0
High	18	31.0
<b>Eggs</b>		
Low/moderate	46	79.3
High	12	20.7
<b>Energy (zinc-rich) drinks</b>		
Low/moderate	49	84.5
High	9	15.5

#### **Breast milk zinc concentrations and associated factors:**

In this study, the median breast milk zinc level was 0.091 [0.063, 0.15] mg/100 mL. Mann-Whitney U tests showed that breast milk zinc concentrations were higher among the lactating women who were younger than 30 years, lived in urban areas, were employed, and reported high consumption of zinc-rich drinks. The detailed differences in the breast milk zinc concentrations are shown in Table 4.

**Table (4):** Differences in breast milk zinc concentrations.

Variable	n	%	Breast milk zinc level (mg per 100 mL)			p-value
Demographic variables			Q1	Median	Q2	
Maternal age (years)						
< 30	30	51.7	0.08	0.10	0.16	0.045
≥ 30	28	48.3	0.06	0.07	0.13	
Place of residence						
Urban	31	53.4	0.08	0.10	0.16	0.017
Rural	27	46.6	0.06	0.07	0.13	
Educational level						
School	19	32.8	0.06	0.08	0.13	0.375
University	39	67.2	0.07	0.10	0.14	
Employment status						
Unemployed	47	81.0	0.06	0.08	0.14	0.046
Employed	11	19.0	0.09	0.11	0.19	
Lactation period (months)						
< 6	39	67.2	0.07	0.10	0.15	0.062
≥ 6	19	32.8	0.05	0.07	0.14	
Gender of the child						
Male	22	37.9	0.06	0.07	0.12	0.229
Female	36	62.1	0.07	0.10	0.16	
Signs of zinc deficiency						
Loss of appetite						
No	51	87.9	0.07	0.10	0.15	0.124
Yes	7	12.1	0.05	0.06	0.10	
Sleepiness/lethargy						
No	52	89.7	0.07	0.09	0.15	0.176
Yes	6	10.3	0.05	0.06	0.13	
Changes in weight						
No	47	81.0	0.06	0.09	0.15	0.558
Yes	11	19.0	0.06	0.08	0.12	
Hair loss						
No	51	87.9	0.06	0.10	0.15	0.299
Yes	7	12.1	0.06	0.07	0.08	

Variable	n	%	Breast milk zinc level (mg per 100 mL)			p-value
Demographic variables			Q1	Median	Q2	
Easily broken nails						
No	50	86.2	0.06	0.10	0.15	0.162
Yes	8	13.8	0.05	0.08	0.10	
Immune diseases/issues						
No	51	87.9	0.07	0.10	0.15	0.088
Yes	7	12.1	0.04	0.05	0.10	
Dermatologic issues (dryness/scaling)						
No	47	81.0	0.06	0.10	0.15	0.174
Yes	11	19.0	0.05	0.07	0.12	
Consumption of sources of zinc						
Red meat/poultry						
Low/moderate	37	63.8	0.06	0.08	0.12	0.042
High	21	36.2	0.08	0.13	0.18	
Milk and dairy products						
Low/moderate	36	62.1	0.06	0.08	0.12	0.089
High	22	37.9	0.06	0.10	0.18	
Whole grains						
Low/moderate	39	67.2	0.06	0.08	0.11	0.065
High	19	32.8	0.06	0.15	0.18	
Fish and seafood						
Low/moderate	40	69.0	0.06	0.08	0.13	0.069
High	18	31.0	0.07	0.10	0.20	
Eggs						
Low/moderate	46	79.3	0.06	0.08	0.13	0.084
High	12	20.7	0.08	0.12	0.22	
Energy (zinc-rich) drinks						
Low/moderate	49	84.5	0.06	0.08	0.13	0.047
High	9	15.5	0.10	0.12	0.26	

Q1: First quartile, Q3: third quartile.

## Discussion

Arguably, maintaining zinc concentrations within the recommended levels can save lives and prevent a considerable number of deaths of infants [8, 10]. Previous studies have measured blood zinc levels among lactating women [13]. In this study, breast milk zinc concentrations were among lactating Palestinian women. Additionally, associations between breast milk zinc levels, dietary zinc intake, and signs and symptoms of zinc deficiency were also assessed. Compared to blood, breast milk could better mirror the zinc available to the breastfed infants. Therefore, assessing breast milk zinc concentrations could provide a more accurate estimate of infant's exposure to zinc received through breastfeeding. Moreover, sampling breast milk is generally less invasive compared to sampling blood, notably, when breast milk zinc concentrations can be used as a reliable indicator of maternal zinc status. Although sampling blood could be informative, however, blood zinc concentrations provide little information on the dynamic nature of zinc transferred to the breastfed infants.

Despite differences in the analytical methods used, the breast milk zinc concentrations quantified in this study were within the range of levels that were previously reported among non-malnourished lactating women in Palestine and elsewhere [2, 13, 15]. Moreover, the breast milk zinc concentrations quantified in this study were within those reported in a previous systematic review and meta-analysis [12]. It is noteworthy to mention that breast milk zinc concentrations can vary between hindmilk and foremilk as well as by the approaches used to collect breast milk samples, time of collecting the samples, methods used to prepare the samples for analysis, and the analytical approaches [2, 13, 15, 19, 20]. The findings obtained in this study indicated that the analytical method used in this study was robust and suitable to quantify breast milk zinc concentrations.

In this study, the breast milk zinc concentrations were significantly lower among older lactating women. These findings were consistent with those previously reported on the postpartum decline in breast milk zinc concentrations [2, 15, 19-21]. On the other hand, some previous studies did not find a link between maternal age and breast milk zinc concentrations [22]. Similarly, being employed and living in urban areas were positively associated with higher zinc concentrations in breast milk. These findings were consistent with those reported among Palestinian and Latvian lactating women [2, 15]. These findings might indicate that living in favorable conditions could be associated with higher breast milk zinc concentrations.

In this study, breast milk zinc concentrations were generally lower among the lactating women who reported signs and symptoms of zinc deficiency compared to the women who did not report signs and symptoms of zinc deficiency. However, the differences were not statistically significant. Probably, the lack of detecting significant differences could be attributed to the small number of lactating women who reported signs and symptoms of zinc deficiency. Therefore, future studies should include a larger sample of women with signs and symptoms of zinc deficiency. Interestingly, the lactating women who reported high consumption of red meat and poultry and those who reported high consumption of zinc-rich drinks had significantly higher breast milk zinc concentrations. Although the breast milk zinc concentrations were generally higher among the women who reported high consumption of dietary zinc sources, the differences were not statistically significant. Probably, this could be attributed to the small number of women who were included in this study. Red meat and poultry are important sources of dietary zinc. Similarly, the consumption of zinc-rich drinks was previously shown to be associated with zinc concentrations among lactating women [2, 13]. The findings reported in this study might indicate that decision and policymakers in the Palestinian healthcare system, nutritionists, lactating advisors, pediatricians, gynecologists, and other healthcare providers

should recommend and ensure that lactating women meet their daily zinc allowance [9, 10, 15, 20].

**Limitations of the study:** While interpreting the findings reported in this study, the following limitations might be considered. First, the sample size used in this study was relatively small. The inclusion of a larger sample should have allowed the detection of more significant associations. However, the sample size used in this study was comparable to those used in some previous studies to assess zinc levels in breast milk [12]. Second, foremilk samples were analyzed in this study. A comparison between the quantities of zinc in foremilk and hindmilk should produce interesting findings. Third, this study was merely observational. It could have been more interesting to conduct an interventional study probably by providing women with zinc supplements. Third, some of the data collected in this study were reported. These data could be susceptible to desirability and recall bias. Fourth, breast milk zinc concentrations were quantified using a graphite furnace atomic absorption spectrophotometric method. Compared to inductively coupled plasma mass spectrometry, atomic absorption spectrophotometry is less sensitive. Finally, zinc levels were not assessed in the blood of the lactating women. Future studies should consider assessing zinc deficiency in pregnant and lactating women.

## Conclusion

Breast milk zinc concentrations were affected by some demographic variables of the lactating women. These variables included, demographics, living conditions, and consumption of zinc-rich sources. The findings of this study can be informative to decision and policymakers in the Palestinian healthcare system who might need to design appropriate interventions to optimize breast milk zinc concentrations. Moreover, dieticians, lactation consultants, and other healthcare providers should screen for signs and symptoms of zinc deficiency and educate/counsel lactating women on the importance of maintaining adequate breast milk zinc levels and consuming zinc-rich sources. Larger studies are still needed to investigate the link between breast milk zinc concentrations and signs and symptoms of zinc deficiency. More studies are still needed to assess the impact of zinc supplements on breast milk zinc concentrations.

## Disclosure Statement

- **Ethics approval and consent to participate:** The Institutional Review Board (IRB) of An-Najah National University provided ethical approval to conduct this study. The IRB approval number given for this study was IRB: #Nsg. Oct. 2023/30. All women who donated breast milk samples gave written informed consent.
- **Consent for publication:** Not applicable.
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methodology, validation, visualization, and writing review & editing. **Dalia Nasser:** data curation, formal analysis, investigation, methodology, validation, visualization, and writing review & editing. **Eman Bdier:** data curation, formal analysis, investigation, methodology, validation, visualization, and writing review & editing. **Raneem Zarour:** data curation, formal analysis, investigation, methodology, validation, visualization, and writing review & editing. This work was based on the graduation project of Mays Hussien, Razan Daraghmah, Dalia Nasser, Eman Bdier, and Raneem Zarour, Medical Laboratory Program, Faculty of Medicine and Health Sciences, An-Najah National University Hospital, Nablus, Palestine.

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