ORIGINAL RESEARCH

A Study of Correlation between the Calcium levels in Breast milk and the Anthropometric Measurements of infants up to 6-months of Life in a Tertiary Care hospital: A Cross-sectional study

Neelam Patil[1], Avinash Jadhao[2], Sandip Lambe[3*], Kanchan Lambe[4]

Abstract

Background: Breast milk an optimal source of nutrition for infants, provides exclusive benefits for their health and growth. It contains specific components that are not found in other foods and that support the development of the child, particularly the height. One of these components is calcium, which is essential for bone formation and function. Calcium intake from breast milk can influence the mineralization of the new bone tissue and the activity of osteoblasts, the cells that produce bone.

Objectives: The researchers proposed that calcium levels in breast milk would change based on how long the mothers exclusively breastfed their babies and that higher calcium levels would correspond to longer body length of infants from Birth to 6-months of age.

Patients and Methods: Colostrum is the first milk produced by mothers after giving birth. This study collected colostrum samples from One Hundred mothers (50 undernourished and 50 well-fed) who delivered at Hospital. The samples were obtained on day 3, month 1, and then every month until month 6 postpartum. The mothers expressed 10 mL of colostrum into acid-washed test tubes. The study analyzed colostrum, which is the first milk produced by mothers after giving birth. The researchers used a flame atomic absorption spectrophotometer (GBC, 904) to measure the calcium levels in the digested samples. The measurements were done at the environmental assessment division of tertiary care hospital and reported in mg/L.

Results: The results indicated a negative correlation relationship between the calcium content of maternal milk and the growth of babies in terms of height during the first half year of life.

Conclusion: Body length is a complex outcome that reflects multiple aspects of growth, not just calcium status. Other factors that may affect body length include genetic factors, such as parental height, and environmental factors, such as other nutrients and hormones.

Keywords: Anthropometry, Breast milk, Breastfeeding, Calcium, Colostrum

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Introduction

Breast milk is a complex fluid that consists of fat droplets dispersed in a mixture of protein, lactose, and minerals that is produced by the mammary glands of the mother and serves as the primary nourishment for the infant. Breastfeeding exclusively and starting as soon as possible after birth, as well as continuing for at least two years, can enhance the infant's immune system.[1] The WHO, United Nations Children's Fund (UNICEF) and Ministry of Health (MoH) of India recommend breast milk as the optimal basis of nourishment for infants till 6-months of life, because it provides essential micro- and macronutrients that are necessary for the growing and physical progress of infants.[2] Breastfeeding exclusively for the first 6-months of life is a crucial period for optimal growth and development of infants, and continuing breastfeeding until 2-years of age or beyond is recommended as a standard practice for infant feeding.[3] Exclusive breastfeeding provides essential nutrients and immunity that are vital for the health and survival of infants.[4] A common sign of nutritional problems in children is stunted growth, which means that children who are malnourished are shorter and lighter than their peers. Chronic and severe malnutrition can impair growth and development, and affect cognitive and physical abilities.[5] The average length of a newborn is 45-50 cm, and according to the National Center for Health Statistics (NCHS) curve, the baby's measurement should

[1]Department of Biochemistry, Topiwala National Medical College and Nair Hospital, Mumbai, Maharashtra, India

[2]Department of Biochemistry, Seth G.S. Medical College and KEM Hospital, Mumbai, Maharashtra, India

[3]Department of Biochemistry, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra, India

[4]Department of Oral Medicine and Radiology, SMBT Institute of Dental Sciences and Research, Nashik, Maharashtra, India

Corresponding Author: Sandip Lambe (**Email:** drsandiplambe@gmail.com)

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increase by a factor of about 2.5 centimetres each month.[6] A cohort type research which was performed on children from natal to 24-months of time of life in a Bangladesh slum area found that maximum number of the petite children had consumptions of micronutrients like vitamins A, E, B complex, calcium, iron, and zinc which were inadequate or less than the normal recommended value which was recommended internationally.[7] A comparable study was also steered by Arsenault JE *et al*. in 2013, which stated that nearly all children aged 24-48 months and women had an insufficient average consumption of micronutrients than recommended. The amount and the quality of macronutrient consumption affects plasma insulin growth factor I (IGF-I) levels in addition to the bone matrix proteins & growth factors that have an important part in bone development. Furthermore, in the Lancet Series in 2013, it was enlightened about numerous micronutrients that have a very significant part in children's linear growth, specifically vitamin A, zinc, iron, iodine, calcium and phosphorus so that they are very vitally required in the physique development to avert development failure.[8] Throughout development, the necessity for bone mineralization is very high, less calcium intake will lead to little mineralization of the grid of new bone stores and osteoblast brokenness. Calcium inadequacy will affect the direct growth, if the calcium content in the bone is under half of the ordinary ingredient.[9] Calcium is very vital for the body, so calcium consumption must be adequate from birth to help the baby's bone development.[1] The small intestine absorbs calcium from the food we consume and sends it to the blood, where it attaches to a protein called albumin. The amount of calcium in the blood depends on how much albumin there is. Calcium is stored in the bones as crystals that combine with phosphorus, forming calcium phosphorus.[10]

The aim of this study was to investigate the relationship between calcium in breast milk and infant growth. The researchers hypothesized that calcium levels in breast milk would vary depending on the duration of exclusive breastfeeding and that higher calcium levels would be associated with greater body length of infants from Birth to 6-months of age. The study was carried out at the Human Milk Bank and Research Centre, Department of Neonatology in a Tertiary Care Centre. Breast milk samples were obtained from mothers with different exclusive breastfeeding durations and analyzed for calcium content. The body length of their infants was measured from birth to 6-months of age and correlated with the calcium levels in breast milk.

Material And Methods

This research aimed to examine the calcium content in human milk and how it relates to the growth of newborns (assessed by anthropometric measures) and maternal factors. The research design was cross-sectional and involved lactating mothers and their infants in a tertiary care hospital. The data were collected from January to June. The mothers were selected by simple random sampling and their milk samples were sent to the laboratory of a tertiary care hospital for calcium analysis.

The study included mothers who had a normal vaginal delivery of a healthy baby at full term and who were breastfeeding their infants. The study excluded mothers who had a cesarean section or an assisted delivery, who had any medical or obstetric condition, or who were taking any medication. The following data were collected from the mothers using a printed form: age, parity, hemoglobin level, weight, height, waist-to-hip ratio, and nutritional grade. The weight of the mothers was measured by means of a weighing scale (Libra). The tallness of the mothers was measured using a height chart. The hemoglobin level was determined by Sahli's method.

The samples were colostrum, the first milk secreted by mothers after childbirth. The study involved 100 mothers (50 with malnutrition and 50 with adequate nutrition) who gave birth at Hospital. The colostrum samples were taken on the third day, one month, and then monthly until six months postpartum. The mothers manually expressed 10 mL of colostrum into test tubes that had been cleaned with acid. The lab equipment used for sample ashing, analysis and storage were also treated with acid and rinsed with distilled and double-distilled water before use.

The colostrum and breast milk samples were digested with a mixture of nitric acid (HNO_3) and perchloride acid ($HClO_4$) in a ratio of 5:3. The volume of the acid mixture required for complete digestion of 2 mL milk was 4 mL. A blank sample containing the same volume of acid mixture was also processed along with each batch of milk samples to account for any interference from the reagents. The concentrations of calcium in the digested samples were measured by flame atomic absorption spectrophotometer (GBC, 904) at the laboratory of a tertiary care hospital. The results were expressed in mg/L.

Statistical analysis

Statistical data was analysed using the GraphPad Prism (United States). An unpaired sample t-test was used to compare the means of the two groups. A simple linear regression scale was used for two group's comparison. A *p-value* of ≤ 0.05 was considered statistically significant.

Results

Demographic profile

Table 1 shows the demographic characteristics of the mothers in the two groups, including age, weight, height, weight / height ratio and haemoglobin concentration. The weight/ height ratio was used as a criterion for maternal malnutrition, with a cut-off point of 0.3. The mothers in the study group were divided into well-nourished and malnourished subgroups based on this ratio. The mean weight, height and haemoglobin levels of the mothers in each subgroup are presented in Table 1. The malnourished mothers had

Table 1: Maternal demographic profile.

Parameter	Well-nourished (n = 50)	Malnourished (n = 50)
Age of mothers (years)	23.8 ± 2.99	22.82 ± 2.41
Weight of mothers (weight)	*49.4 ± 5.91	45.5 ± 2.17
Height of mothers (cm)	*155.34 ± 4.54	148.94 ± 3.17
Weight/height ratio (kg /cm)	*0.35 ± 0.042	0.28 ± 0.022
Haemoglobin (g%)	*11.37 ± 2.03	9.9 4 ± 0.75

Table 2: Comparison of Calcium Levels in Breast Milk between well-nourished and malnourished mothers (mg/L).

Calcium Levels in Breast Milk	Well-nourished (n = 50)	Malnourished (n = 50)
Third Day	259.31 ± 75.66	250.22 ± 61.55
1-month	258.41 ± 57.36	250.09 ± 51.34
2-months	258.25 ± 25.16	249.22 ± 12.25
3-months	253.66 ± 35.76	244.22 ± 43.86
4-months	252.34 ± 75.62	243.82 ± 76.92
5-months	252.13 ± 75.50	243.22 ± 61.77
6-months	249.21 ± 75.64	242.21 ± 41.55

significantly lower weight, height and haemoglobin levels than the well-nourished mothers.

Table 2 compares the calcium content of breast milk from well-nourished and malnourished mothers. Calcium is an important mineral for bone development and maintenance. The calcium content of breast milk did not differ significantly between the two groups of mothers, suggesting that maternal nutrition status did not affect the calcium transfer to breast milk.

Tables 3 and 4 highlight the differences in weight and height progression among infants of well-nourished and malnourished mothers over the first six months of life. Infants of well-nourished mothers consistently showed significantly higher weight gains compared to those of malnourished mothers, with the difference becoming more pronounced as the months progressed. A similar trend was observed for height measurements, as depicted in Table 4, where infants of well-nourished mothers exhibited greater increments in body length throughout the study period. Despite the consistent increase in weight and height across both groups, the findings demonstrated a significant negative correlation between the calcium levels in breast milk and both weight and height gains, suggesting that other factors, including maternal nutrition and environmental influences, may play a more dominant role in determining infant growth during the first six months of life.

Table 3: Comparison of changes in weight between the babies of well-nourished and malnourished mothers (gms).

Calcium Levels in Breast Milk	Well-nourished (n = 50)	Malnourished (n = 50)
Third Day	2813.0 ± 334.84	2568 ± 330.57
1-month	3418 ± 332.55	2979.0 ± 364.93
2-months	4009 ± 407.54	3365.6 ± 442.9
3-months	4683 ± 560.67	3637.8 ± 699.7
4-months	5339 ± 675.4	4057 ± 609.6
5-months	5907 ± 778.7	4380.0 ± 737.83
6-months	6555 ± 786.2	4678.4 ± 797.98

Table 4: Comparison of changes in height of the babies between well-nourished and malnourished mothers (Cms).

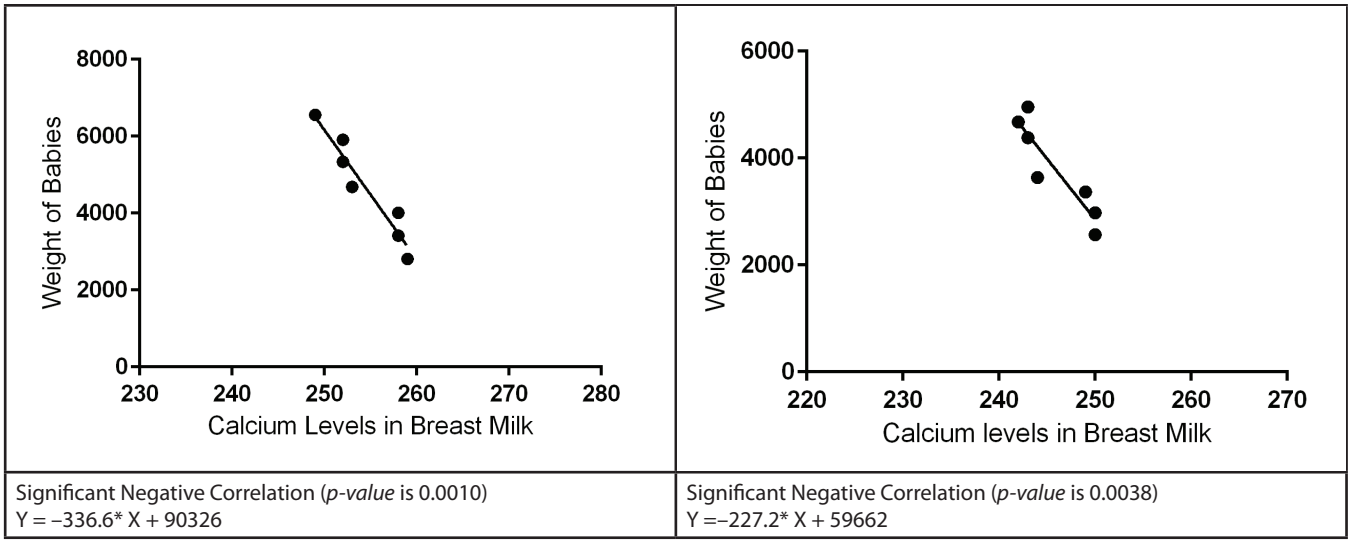
Calcium Levels in Breast Milk	Well-nourished (n = 50)	Malnourished (n = 50)
Third Day	49.26 ± 1.93	48.65 ± 2.28
1-month	51.58 ±1.83	50.26 ± 2.39
2-months	54.07 ± 2.20	51.68 ± 2.63
3-months	56.34 ± 2.60	53.13 ± 2.93
4-months	57.42 ± 2.85	53.56 ± 7.73
5-months	60.64 ± 3.12	56.17 ±3.61
6-months	62.80 ± 3.08	58.23 ± 2.53

Table 5: Comparison of changes in chest circumference of babies between well-nourished and malnourished mothers (Cms).

	Well-nourished (n = 50)	Malnourished (n = 50)
Third Day	31.13 ± 2.01	30.42 ± 1.88
1-month	33.48 ± 2.38	31.72 ± 2.03
2-months	35.38 ±2.33	33.03 ±2.35
3-months	37.14 ± 3.06	34.04 ± 2.81
4-months	38.52 ± 2.72	35.04 ± 2.81
5-months	39.74 ± 2.43	35.69 ± 2.98
6-months	40.94 ± 2.27	36.30 ± 3.19

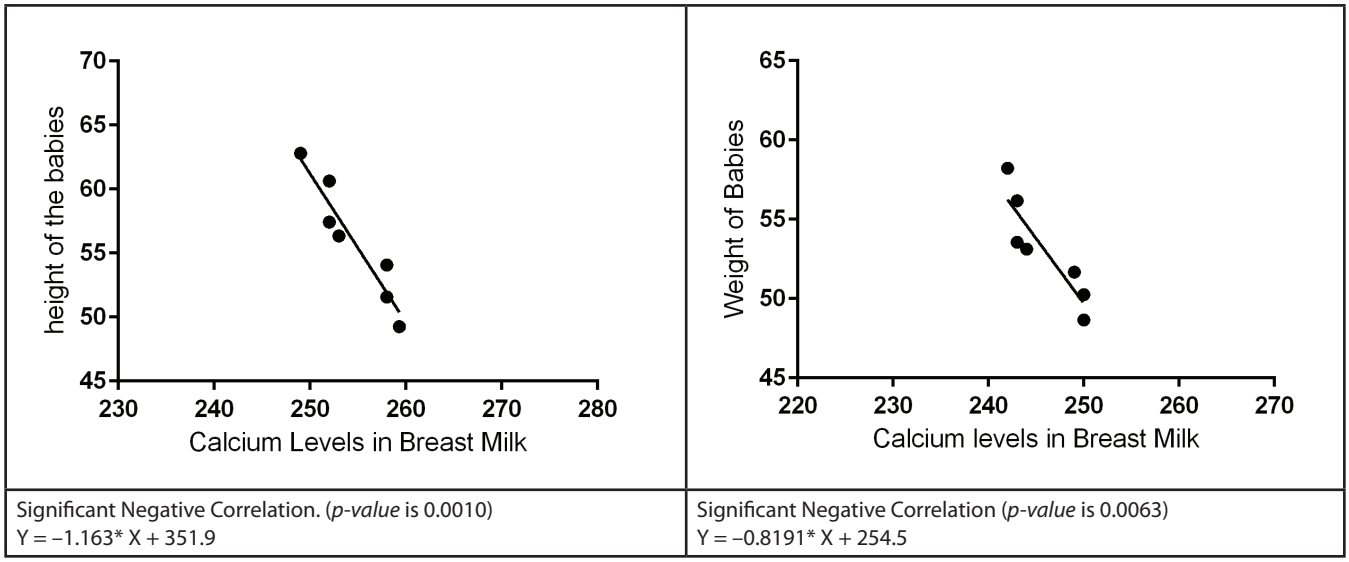
Table 6: Comparison of changes in foot length of babies between well-nourished and malnourished mothers (Cms).

	Well-nourished (n = 50)	Malnourished (n = 50)
Third Day	7.87 ± 0.49	7.58 ± 0.48
1-month	8.22 ± 0.51	7.82 ± 1.15
2-months	8.59 ± 0.53	8.24 ± 1.02
3-months	8.93 ± 0.53	8.51 ± 0.93
4-months	9.28 ± 0.58	8.72 ± 0.85
5-months	9.58 ± 0.66	8.97 ± 0.80
6-months	9.99 ± 0.66	9.19 ± 0.77
Changes	**2.21** ± 0.71	**1.61** ± 1.01



Significant Negative Correlation (*p-value* is 0.0010) Y = –336.6* X + 90326	Significant Negative Correlation (*p-value* is 0.0038) Y =–227.2* X + 59662

Figure 1: (A) Correlation between Calcium Levels in Breast Milk of well-nourished mother with weight of their babies. **(B)** Correlation between Calcium Levels in Breast Milk of malnourished mother with weight of their babies.



Significant Negative Correlation. (*p-value* is 0.0010) Y = –1.163* X + 351.9	Significant Negative Correlation (*p-value* is 0.0063) Y = –0.8191* X + 254.5

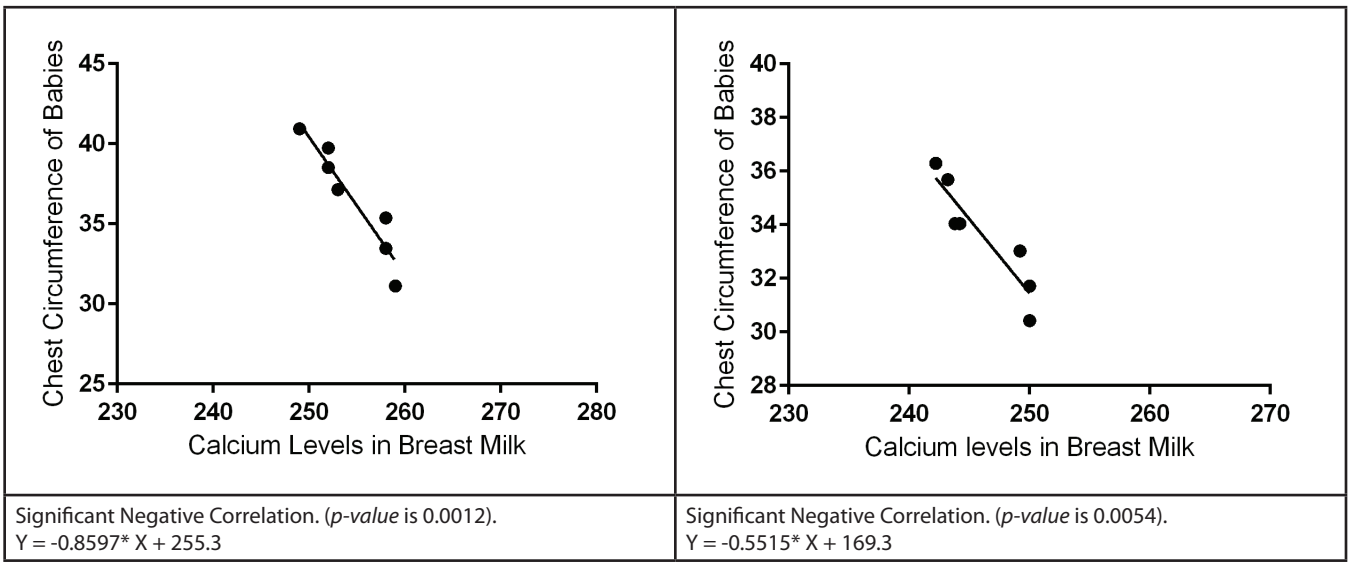
Figure 2: (A) Correlation between Calcium Levels in Breast Milk of well-nourished mother with height of their babies. **(B)** Correlation between Calcium Levels in Breast Milk of malnourished mother with height of their babies.

Discussion

Breast-feeding practices vary widely between mothers in the emerging world and those in affluent countries. Mothers in the developing world tend to breast-feed on call for extended periods and use weaning nourishments as a supplement, while mothers in affluent countries tend to breast-feed less frequently and use weaning foods as a substitute. However, the intra-subject disparities in breast-milk calcium concentrations were comparable to those reported from the developed countries (5-8), regardless of the phase of feed or time of day. This indicates that demonstrative milk samples can be simply gained by means of a modest protocol. The duration of lactation had a significant effect on breast-milk calcium concentrations in both urban and rural populations. Calcium concentrations stayed stable during the first 3-months of lactation, followed by a gradual decline that was observed in our study.

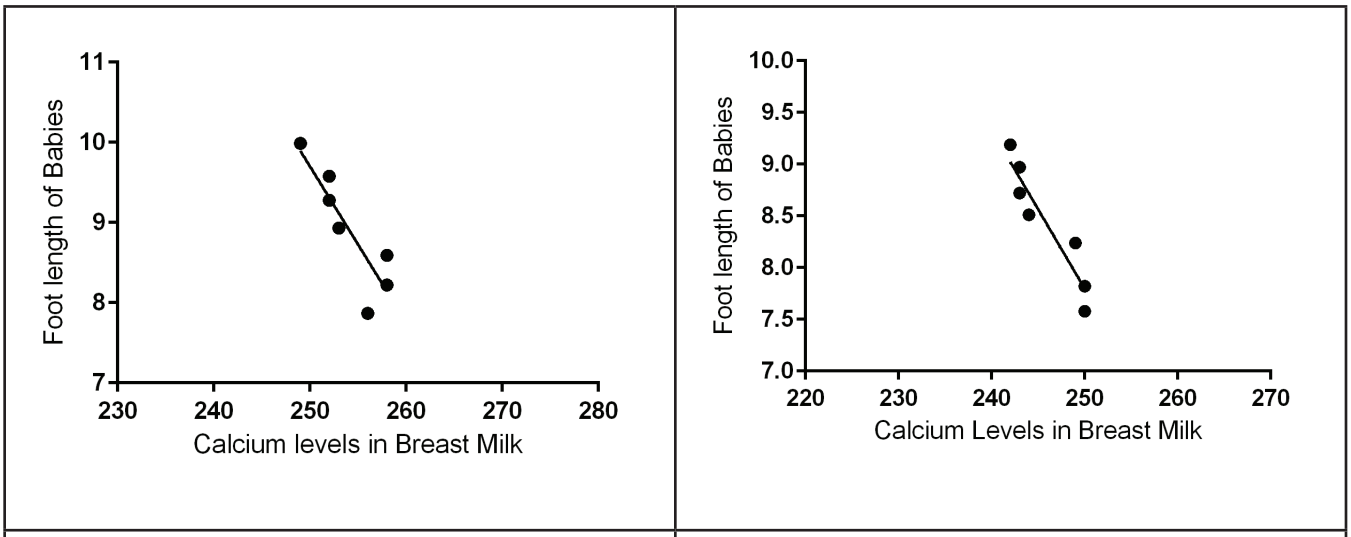
The Association amongst Calcium Levels in Breast Milk and Body Length for 6-Months Old Babies

Breast milk is the optimal source of nutrition for infants, as it provides a balanced mix of nutrients that support their growth and development both in the short and long term. After six months of age, infants start to receive complementary foods in addition to breast milk, which may vary in their calcium content and quality. Calcium is an essential mineral that plays a key role in building and maintaining bone and tooth health.[12]



Significant Negative Correlation. (*p-value* is 0.0012). Y = -0.8597* X + 255.3	Significant Negative Correlation. (*p-value* is 0.0054). Y = -0.5515* X + 169.3

Figure 3: (A) Correlation between Calcium Levels in Breast Milk of well-nourished mother with Chest Circumference of their babies. **(B)** Correlation between Calcium Levels in Breast Milk of malnourished mother with Chest Circumference of their babies.



Significant Negative Correlation (*p-value* is 0.0017) Y = –0.8235* X + 246.8	Significant Negative Correlation (*p-value* is 0.0015) Y = –0.1534* X + 46.15

Figure 4: (A) Correlation between Calcium Levels in Breast Milk between well-nourished with Foot length of babies of well-nourished mother. **(B)** Correlation between Calcium Levels in Breast Milk between well-nourished with Foot length of babies of mal nourished mother.

This study aimed to examine the correlation between calcium levels in breast milk and the body length of infants who were exclusively or partially breastfed for six months. The findings showed that there was a significant negative correlation between calcium levels in breast milk and the body length of infants from birth to six months of age.

One possible explanation for this result is that calcium intake and growth are influenced by many other factors besides breast milk, such as the quantity and quality of complementary foods, the total calcium intake from all sources, and the individual needs of each infant for bone mineralization. Low calcium intake from breast milk and complementary foods may result in low total calcium intake, which may compromise the infant's growth potential. Moreover, body length is a complex outcome that reflects multiple aspects of growth, not just calcium status. Other factors that may affect body length include genetic factors, such as parental height, and environmental factors, such as other nutrients, hormones, and infections.[13]

Therefore, calcium in breast milk alone cannot predict the body span of infants at six months of age. Calcium and bone metabolism are closely linked and interdependent, but they are also modulated by many other factors that need to be considered in future research.[14]

Calcium is an essential nutrient for health, especially for bone development and maintenance. Calcium deficiency can lead to growth problems and other health issues related to calcium's role in the body. Bones are mainly composed of calcium, so getting enough calcium from food sources is important for bone health and strength. This is consistent with Hardinsyah's study, which found no significant relationship between calcium from milk and height. This is because height is influenced by other factors besides calcium, such as growth hormone, IGF-I, genetic factors, physical activity and exercise. In this study, there was no correlation between parental height and child height. Saptawati's research also reported that 15.7% of the subjects were stunted, more than 80% of the subjects had low calcium intake, and there was no association between calcium intake and height indicators.[12]

Another study by Endika showed that among 30 infants aged 4-6 months, 15 were exclusively breastfed and 15 received formula milk, there was no difference in body length gain between the two groups using an independent t-test ($p=0.355$).[15] There was also no significant relationship between calcium levels in breast milk and body length at 6-months or 12-months of age using Spearman correlation test.

Conclusion

The calcium content of breast milk from mothers with adequate or inadequate nutrition was normal from day 3 to 6 months postpartum. The infants' growth indicators did not show significant differences during this period. There was a significant negative correlation between the breast milk calcium and the infants' growth indicators until 6-months of age. Body length is a complex outcome that reflects multiple aspects of growth, not just calcium status. Other factors that may affect body length include genetic factors, such as parental height, and environmental factors, such as other nutrients and hormones.

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