Effect of maternal and infant vitamin D supplementation on vitamin D levels of breastfed infants

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SUMMARY: Buğrul F, Devecioğlu E, Özden T, Gökçay G, Ömer B. Effect of maternal and infant vitamin D supplementation on vitamin D levels of breastfed infants. Turk J Pediatr 2013; 55: 158-163.

The aim of our study was to investigate the effect of maternal vitamin D_3 (400 U/day) supplementation on breastfed infants at 6 months of age. Mothers (n=96) were enrolled within 1 month after birth and assigned to the 400 IU/day regimen or no vitamin D_3 supplementation for 6 months. All infants received 400 IU/day of vitamin D_3 and were exclusively breastfed until 4 months of age. Of all mothers, 22.2% had vitamin D levels above 20 ng/ml initially. At the end of the study, vitamin D levels of mothers and their infants were similar in both groups. Thirteen percent of the infants in the intervention group and 20.5% in the control group had vitamin D levels below 12 ng/ml. Serum 25-hydroxyvitamin D (25(OH)D) concentrations at 6 months had increased significantly in mothers in the intervention group. Lactating mothers and their children need vitamin D supplementation but further studies are required with higher doses.

Key words: breastfed infants, lactating women, maternal vitamin D supplementation, vitamin D deficiency.

Vitamin D deficiency (VDD) in mothers and their breastfed infants is a significant health problem worldwide. Severe VDD leads to rickets in children. Supplementation of breastfed infants with recommended doses of vitamin D may not be enough to prevent VDD in high-risk populations. Recent studies have shown that occurrence of VDD can also be associated with an increased incidence of chronic diseases including many types of cancer, cardiovascular disease, diabetes, obesity, and autoimmune diseases, as well as several bacterial and viral infections[1]. Thus, an adequate intake of vitamin D in infancy and childhood has been the main subject in many researches.

Many studies have shown a strong relation between maternal and neonatal 25-hydroxyvitamin D (25(OH)D) concentrations[2-4]. Studies performed in the last decade detected moderate-to-severe VDD in 46-80% of pregnant and lactating women in different regions of Turkey[4-6]. Similar results were also reported from different parts of the world[7-9]. Scientific data related to vitamin D supplementation during lactation are scarce. The daily recommended intake for vitamin D during lactation has been arbitrarily set at 400-600 IU/day (10-15 μg/d).

However, there is no scientific evidence supporting this current recommendation. Therefore, the investigators focused on maternal vitamin D supplementation, and a recent study in a small group showed that a maternal supplementation of 6400 IU/day during lactation could achieve substantial progress in improving the vitamin D status of mothers and their infants without any adverse effect[10].

The aim of this study was to investigate the factors affecting vitamin D levels of the mothers and to evaluate the effect of 400 IU/day maternal vitamin D supplementation on vitamin D levels of breastfed infants at 6 months of age.

Material and Methods

This prospective, controlled intervention study was conducted in the Well Child Clinic of the

This research was supported by İstanbul University Research Fund (no: 12640).

Social Pediatrics Department of the İstanbul Faculty of Medicine, İstanbul University, between May 2010 and March 2011. The study was approved by the Ethical Committee of the Medical School. Full-term newborns with 37 completed weeks of gestation, birth weight ≥2500 g and ≤4000 g with an uneventful perinatal history constituted the study population. Newborns with chromosomal anomalies, genetic diseases or congenital malformations were not included. Fully lactating mothers within their first postpartum month and their infants were selected as eligible for the study if the mothers planned to continue full breastfeeding for 6 months.

Assuming that the mean vitamin D level of the intervention group will be 10% higher than the control group, with a confidence interval of 95%, and given an alpha level of 5% and a power of 80%, we needed to obtain data on 50 infants in each group. Subjects were recruited between May and October 2010. During this period, 216 newborns applied to our clinic; 200 of them fulfilled the inclusion criteria and half of them gave consent for the study. The 50 mother-newborn pairs who applied to our clinic between May 2010 and July 2010 constituted the intervention group and 50 mother-newborn pairs who applied between August 2010 and October 2010 were in the control group. At the beginning of our study, there was no official recommendation for routine vitamin D supplementation during pregnancy and lactation in our country. The women were taking 400 IU/day vitamin D_3 supplementation during pregnancy and lactation if recommended by their physician. We aimed to evaluate the effect of this standard supplementation on vitamin D levels of the breastfed infants. The mothers in the intervention group received 400 IU vitamin D3 per day in our study. The mothers in the control group were asked not to take any supplementation. Mothers in both groups were instructed to give 3 drops (400 IU vitamin D_3) per day of commercially available oil emulsion (Devit-3, Deva) to their infants.

A detailed questionnaire regarding the mother's medical, family and demographic information was completed. Data about the use of prenatal vitamin supplements were also gathered. Mothers were asked about the extent of their concealed clothing and daily exposure to sunlight. Concealed clothing was defined as face open, head and arms covered. Sunlight exposure was defined as going out to open air regularly with the child for at least 15 minutes each day. All data were collected from mothers by face-to-face interviews. At the beginning of the study, approximately 5 ml of venous blood was drawn from all mothers. All mother-infant pairs were followed up every month for 6 months. Six infants from the control group (Withdrawal [n:1], nephrolithiasis [n:1], starting formula [n:4]) and four infants from the intervention group (withdrawal [n:3], metabolic disease [n:1]) were excluded from the study during the follow-up period.

At the end of the follow-up period, 5 ml of venous blood from all mothers and 4 ml from all infants were drawn for estimation of 25(OH) D level. All blood samples were wrapped by a carbon paper to protect from sunlight. The blood samples were labeled and centrifuged immediately. The serum was separated and samples were stored at -20°C until analysis. Thermo Finnigan spectra high-pressure liquid chromatography (HPLC) system (USA) with RECIPE kit (Germany) was used for the estimation. The coefficient of variation (CV) of Thermo Finnigan spectra HPLC system was 3.1%. VDD was defined as a serum 25(OH)D level <12 ng/ml and vitamin D insufficiency as a 25(OH)D level between 12 ng/ml and 20 ng/ml.

The NCSS 2007 statistical program was used to analyze the results. Statistical significance was assessed for discrete variables by χ^2 analysis and for continuous variables by the t test, Kruskal-Wallis test and Mann-Whitney U test. Multivariate logistic regression analyses were used to determine the factors affecting vitamin D levels. This research was supported by İstanbul University Research Fund (no: 12640).

Results

Mean initial vitamin D level of all mothers was 13.5 ± 8.6 ng/ml. Of all mothers, 22.2% had vitamin D levels above 20 ng/ml. Mothers who regularly covered their heads and their arms had significantly lower levels (p=0.041) and those who had exposure to sunlight more frequently than three days a week had high levels of vitamin D (p=0.020), as seen in Table I. Concealed clothing was found to be the most important factor affecting the maternal vitamin D level according to the results of multivariate logistic regression analyses (Table II).

Table I. Assessment of Factors of Possible Significance in Influencing Maternal Vitamin D Levels (Mean±SD)

Factors (n)	Vitamin D level (95% CI)	p
Mother's sun exposure		
1-2 days/week (18)	9.4±4.6 (2.9-22.4)	0.020
≥3 days/week (72)	14.6±9.1 (2.0-38.6)	
Monthly income		
<1000 TL (5)	8.1±2.1 (5.5-10.2)	
1000-1999 TL (29)	11.9±6.2 (2.9-25.1)	0.128
≥2000 TL (56)	14.8±9.7 (2.0-38.6)	
Mother's employment status		
Yes (40)	15.1±8.9 (4.2-38.2)	0.300
No (50)	12.2±8.2 (2.0-38.6)	
Wearing concealing clothes		
No (48)	15.9±8.8 (4.8-38.6)	0.041
Yes (42)	10.7±7.4 (2.0-38.2)	
Maternal education		
Primary-middle school (27)	11.8±7.7 (2.9-38.6)	0.103
High school and higher (63)	14.2±8.9 (2.0-38.2)	
Parity		
1 (48)	14.5±9.3 (2.7-38.6)	
2 (31)	13.4±8.5 (2.0-32.4)	0.662
3 (9)	9.1±2.1 (6.5-12.7)	
4 (2)	9.4±2.8 (7.4-11.4)	
Mother's age		
20-25 (10)	9.3±5.1 (3.8-20.2)	
26-30 (33)	13.4±9.2 (2.7-38.2)	0.607
31-35 (31)	14.8±8.9 (2.0-38.6)	
≥ 36 (16)	14.1±8.1 (5.5-32.4)	
Exposure to sun in the house		
Yes (82)	13.9±8.7 (2.0-38.6)	0.095
No (8)	9.7±5.8 (3.8-22.4)	

CI: Confidence interval.

The main characteristics of the intervention and control groups are given in Table III. There was no statistically significant difference between the two groups. At the end of the follow-up period, mean vitamin D levels of all mothers who received vitamin D supplementation was 13.1 ± 8.8 ng/ml, not significantly different from those of the control group (10.9 ± 6.6 ng/ml; p=0.188). However, there was an increase in vitamin D levels of the mothers in the intervention group (mean increase = Δ 0.41 ± 9.86), while a decrease was found in the control group (mean decrease = -3.44 ± 10.9 ng/ml). The difference between the increments was significant (p=0.034).

At the end of the study, mean vitamin D levels of infants in the intervention group (35 ± 16.3 ng/ml) was similar (p=0.821) to those of the control group (34.6 ± 21.4 ng/ml). Seventeen percent of the children in the intervention group and 30% in the control group had vitamin D levels below 20 ng/ml at the end of the study (Table IV).

No relationship was found between the mean vitamin D levels of the infants and

Table II. Assessment of Factors of Possible Significance in Influencing Initial Maternal Vitamin D Levels (Multivariate Logistic Regression Analysis)

	Nonstandard coefficient	Standard error	Standard coefficient	t	p
Maternal sun exposure	4.849	3.064	0.238	1.583	0.117
Concealing clothing	-5.369	1.964	-0.314	-2.732	0.008
Maternal education	-1.302	1.018	-0.190	-1.279	0.204
Sun exposure in the house	-0.283	3.563	-0.009	-0.079	0.937

Table III. Comparison of the Main Characteristics in the Two Groups

	Intervention (n=46)	Control (n=44)	p
Gender of infants (%)			
Boys	20 (43.5)	22 (50.0)	0.535
Girls	26 (56.5)	22 (50.0)	
Maternal age (%)			
20-25 years	7 (15.2)	3 (6.8)	
26-30 years	15(32.6)	18 (40.9)	
31-35 years	16(34.8)	15 (34.1)	0.602
≥36 years	8(17.4)	8 (18.2)	
Maternal education (%)			
Primary-middle school	16 (34.8)	11 (25.0)	0.352
High school and higher	27 (58.7)	33 (75.0)	
Monthly income (%)			
<1000 TL	3 (6.5)	2 (4.9)	
1000-1999 TL	16 (34.8)	13 (29.5)	0.885
≥2000 TL	27 (58.7)	29 (65.9)	
Parity (%)			
1	27 (58.7)	21(47.7)	
2	12 (26.1)	19 (43.3)	
3	7 (15.2)	2 (4.5)	0.073
4	0	2 (4.5)	
Availability of sun exposure in the home (%)			
Yes	41 (89.1)	41 (93.2)	0.5
Wearing concealing clothing (%)			
Yes	20 (43.5)	22 (50.0)	0.535
Vitamin D supplementation during pregnancy (%)			
Yes	46 (100.0)	44 (100.0)	1.0
Initial maternal vitamin D level (ng/ml) (mean±SD)	12.7±8.6	14.4±8.6	0.356
Change in growth parameters of infants from birth to 6 months (mean±SD)			
Δ Weight (g)	4480±760	4345±1107	0.178
Δ Height (cm)	16.9±5.6	17.20±0.768	0.768
Δ Head circumference (cm)	8.2±1.5	7.85±1.8	0.370

the characteristics of their mothers (vitamin D supplementation during pregnancy, sun exposure, concealing clothing, and maternal vitamin D levels) in multi-logistic regression analysis. None of the infants developed clinical signs of rickets during the study period.

Discussion

To our knowledge, our prospective, controlled intervention study is the only study in the literature on the effect of 400 IU/day vitamin D_3 supplementation during lactation. Despite recommendations for vitamin D supplementation of at-risk groups, VDD and infantile rickets remain major public health problems in many countries. There is evidence that the current recommendations, particularly for pregnant and nursing women, are inadequate to ensure vitamin D sufficiency in these groups. At the beginning of our study, which was conducted in a Well Child Clinic, while all mothers reported that they took multivitamin supplementation during their pregnancy, only 22.2% of them were found to have vitamin D levels above 20 ng/ml within 1 month of birth. These findings were similar to those reported by Halıcıoğlu et al.[11]. Several studies have shown that VDD was prevalent among pregnant women worldwide[4-9]

Vitamin D status is affected by many factors like skin pigmentation, latitude, dressing

Table IV. Comparison of Vitamin D Levels in Infants in the Two Groups

Vitamin D levels (ng/ml)	Intervention group (n: 46)	Control group (n: 44)	p
<12	6 (13.0%)	9 (20.5%)	
12-19.9	2 (4.4%)	4 (9.1%)	0.38
≥20	38 (82.6%)	31(70.4%)	

codes, season, sunscreen use, air pollution, and obesity[12]. In our study, mothers who regularly covered their heads and their arms had significantly lower levels of vitamin D (Table I), and wearing concealing clothing was found to be the only factor affecting the maternal vitamin D level (Table II). Similar findings were reported in various studies[4,11,13,14]. Mothers who had exposure to sunlight more frequently than three days a week had high levels of vitamin D. This difference was not statistically significant, but in a study with a larger sample size, duration of sun exposure was associated with higher vitamin D levels[15].

Saadi et al.[16] found that maternal and infant serum vitamin D concentrations correlated positively at 3 months, but we could not find such a correlation at 6 months in our study. Saadi et al.[16] also reported that combined maternal (2000 IU/d) and infant (400 IU/d) vitamin D_2 supplementation for 3 months was associated with a three-fold increase in the serum vitamin D concentrations of the infants. We did not find any effect of 400 U/day vitamin D_3 supplementation of mothers on the vitamin D level of the infants at 6 months of age. This may be due to the fact that all infants in this study were receiving regular vitamin D supplementation during their first 6 months. On the other hand, we did not evaluate the vitamin D levels of infants at 3 months of age. In another study, it was reported that the vitamin D levels of exclusively breastfed infants at 1 month of age reflect maternal vitamin D status, but that such a correlation was not found in infants receiving regular vitamin D supplementation[17].

In our study, mean vitamin D levels of the mothers in the intervention group were not significantly different from those of the control group. However, there was a significant decline in the mean vitamin D levels of the mothers in the control group that led us to think that 400 IU/day vitamin D_3 supplementation may prevent the decrease in the vitamin D level during winter. The mean vitamin D level of infants in the intervention group was essentially similar to that of the control group at 6 months, although in the control group, the proportion of infants with vitamin D levels below 20 ng/ml was higher (30%) as compared to the infants in the intervention group (17%). However, this difference was not statistically significant. Our results led us to think that higher doses of vitamin D supplementation may be needed for lactating mothers and their infants. There are few prospective studies in the literature examining the effect of higher doses of vitamin D supplementation during lactation. In a study with a small sample size, Hollis and Wagner[18] found that supplementing mothers with either 2000 or 4000 IU/day vitamin D_2 for a period of 3 months after birth increased the nutritional vitamin D status of both mother and nursing infant, but the increases were small and insufficient. Wagner et al.[19] reported that daily maternal supplementation of 6400 IU/day of vitamin D_3 was sufficient to maintain adequate levels of 25(OH)D in the nursing infant without any supplementation to the infant and that this effect is comparable to that found in mothers receiving a supplement of 400 IU/day and infants receiving a supplement of 300 IU/day of vitamin D_3.

Our study, conducted on 90 mother-infant pairs, is one of the largest studies on vitamin D supplementation of lactating mothers and their infants, but it had some limitations. We did not have any objective observation confirming that the mothers in the intervention group took their vitamin D supplementation regularly. Vitamin D bioavailability is known to be affected in obese individuals[12], but we did not calculate the body mass index of the mothers at that time. Time of day and season are important factors influencing vitamin D formation[20]. The recruitment of the cases was also not random in our study. The mothers were recruited to the intervention group during spring-summer and to the control group during autumn months. We could not evaluate the sunscreen use of the mothers during this time. These factors

may prevent observation of differences between the intervention and control groups regarding vitamin D levels.

In conclusion, our results showed that mothers and their infants living in İstanbul, a city located in a sunny region, were at risk for VDD. Combined maternal and infant vitamin D supplementation with a dose of 400 IU/day in the first 6 months after birth had no influence on the vitamin D levels of the infants, and vitamin D levels below 12 ng/ml persisted in 13% of these infants. Daily vitamin D intake of 10-15 μg/day was recommended recently for the lactating mother in the United States[21]. Our findings showed that this amount of vitamin D supplementation during lactation is not enough to prevent VDD in lactating mothers and their infants. The implementation of higher doses of vitamin D for lactating mothers, as recommended by the Endocrine Society in February 2011, seems to be prudent[22]. Further studies are required with these newly recommended doses in different populations. Meanwhile, mothers should be advised to benefit from sunlight at any opportunity while taking necessary precautions.

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