

# Comparison of Umbilical Cord and Postnatal Fourth Month Serum 25-OH Vitamin D Levels of Late Preterm and Term Infants

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## What is known on this subject?

Vitamin D is one of the fat-soluble vitamin that is steroidal in structure, that can affect the organism throughout life. The effect of vitamin D is not only to maintain bone metabolism by regulating calcium balance but also additional benefits have reported such as to prevent inflammation and regulating the immune system.

## What this study adds?

Although the cord blood vitamin D levels of late preterm infants are reported to be similar in late preterm and term infants, vitamin D deficiency was notably high in both groups.

It was observed that with oral 400 IU vitamin D3 replacement, vitamin D levels increased significantly and returned to normal levels at postnatal 4<sup>th</sup> month.

## ABSTRACT

**Objective:** Our aim was to compare vitamin D levels of late preterm and term babies measured at birth and at postnatal 4<sup>th</sup> month.

**Material and Methods:** One hundred four late preterm infants (group I) and 118 term infants (group II) were enrolled in the study. Maternal age, parity, morbidities related to pregnancy, educational status, sun exposure, dressing style and use of multivitamin supplements were recorded. Gestational age, birth weight, height, head circumference, sex of infants were also recorded. Umbilical cord blood was collected from all participants and cord blood 25-OH vitamin D levels were measured. Oral vitamin D 3 supplementation (400 IU) was started on postnatal 15<sup>th</sup> day for all babies. Vitamin D measurements were repeated at the postnatal fourth month. Serum 25-OH vitamin D concentrations were measured by chemiluminescence assay. The results were evaluated statistically.

**Results:** Mean umbilical cord 25-OH vitamin D levels of groups I and II were 7.6±6.6 ng/mL and 7.5±6.5 ng/mL, respectively (p=0.835). Eighty-four percent of infants in group I and 78% of infants in group II had severe vitamin D deficiency (<10 ng/mL). Cord blood vitamin D levels in both groups did not differ in terms of sun exposure (p=0.595). A statistically significant increase in 25-OH vitamin D levels was seen after vitamin D supplementation in both groups (p<0.05). Also, 25-OH vitamin D levels at postnatal 4<sup>th</sup> month of life between the two groups did not differ (group I 34.4±8.7 ng/mL vs. group II 38.9±12.7 ng/mL; p=0.306).

**Conclusion:** Although the umbilical cord 25-OH vitamin D blood levels of late preterm infants were similar to term infants', a high incidence of vitamin D deficiency in the umbilical cord blood was observed in both groups. Late prematurity did not pose an additional risk factor for vitamin D deficiency. After four months of oral replacement therapy, repeated serum vitamin D-level measurement confirmed significantly increased vitamin levels, almost reaching normal values.

**Keywords:** Vitamin D, late preterm, newborn, neonatology

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## Introduction

Vitamin D is one of the fat-soluble vitamin that is, which is steroidal in structure and can affect the organism. The effect of vitamin D is not only to maintain bone metabolism by regulating calcium balance, but also additional benefits have reported such as to prevent inflammation and regulating the immune system (1,2).

Maternal vitamin D deficiency has been shown to be an important health problem in developing countries recently. It has been reported that there is an inverse relationship between maternal vitamin D deficiency and fetal growth, ossification, enamel hypoplasia, and neonatal Ca balance.

It was shown that there is a direct correlation between cord blood levels and the mother's serum concentrations of 25-OH vitamin D in the first eight weeks of life, whereas sunlight or oral vitamin D3 supplementation is more effective on vitamin D serum levels after that (3).

The daily requirement of vitamin D for term infants has been determined as 400 IU. The amount of vitamin D amount in breast milk can only meet a very small part of this requirement (12-60 IU/liter) (1-3).

Neonates born between 34<sup>th</sup> and 36<sup>th</sup> gestational age are called late preterm. Although not as much as very low birth weight (VLBW) babies, late preterm babies experience sepsis, jaundice, hypoglycemia, respiratory, and feeding difficulties more often and their mortality rates are higher compared to term babies. One can predict that if the factors affecting mortality and morbidity are better determined, problems that may arise in these babies can be better coped with (4,5). Studies on the daily vitamin D requirement and maternal levels of vitamin D of late preterm infants who are chronologically between VLBW and term infants are limited.

In this study, our aim is to compare the umbilical cord serum and 4<sup>th</sup> month vitamin D levels of late preterm and term infants who were born in our clinic. Moreover, it is one of our goals to reveal whether daily vitamin D supplementation is sufficient and how much vitamin D is needed in late preterm babies compared to term babies.

## Material and Methods

Between January 2013 and April 2013 infants who born in our hospital and followed up in the neonatology clinic after birth were enrolled in this study. Babies were divided into two groups as "late preterm babies born at 34 0/7-36 6/7 weeks" and "term babies those born at 37 0/7 and above". Group I was defined as late preterm babies and group II as term babies. A

total of 222 cases, including 104 late preterm cases and 118 term cases, constituted the study group. Babies born before 34 weeks, with known chronic diseases and without parental consent were excluded from the study.

The study was planned in accordance with the Declaration of Helsinki after obtaining permission from the University of Health Sciences Turkey, Zeynep Kamil Maternity and Children's Training and Research Hospital, Ethical Committee (decision no: 034, date: 15.02.2013). Parents of the babies were informed about the study and their consent were obtained. For data collection, information about the mother (age, previous of pregnancies, education level, breast milk usage, and breastfeeding information in the first four months, vitamin usage during pregnancy, sun exposure, preeclampsia), and the baby (gestational week, delivery type, body weight, laboratory, and clinical characteristics) was gathered.

In both groups, blood was drawn from the umbilical cord for vitamin D levels. In both groups, 400 IU vitamin D3 supplement was initiated enterally after the postnatal 15<sup>th</sup> day. Blood samples were drawn again for blood 25-OH vitamin D levels who came for control at the age of four months.

### Gathering and Collecting Blood Samples

1 mL blood sample was taken from the umbilical cord and then later from the antecubital vein of the babies who were in the study, into biochemistry tubes with gel. The samples were centrifuged at 5000 rpm for 5 min at +4 °C. The serums were separated and stored at -30 °C until the day of the study.

### Analyzing Blood 25-OH Vitamin D Levels

Samples were analysed consecutively, on the Liaison device using the DiaSorin kit (Diasorin Inc. Northwestern Ave-Stillwater, USA) by the chemiluminescent immunoassay method at the biochemistry laboratory of the hospital.

If, 25-OH vitamin D levels >32 ng/mL defined as normal, between 20 and 32 ng/mL defined as insufficiency, <20 ng/mL defined as deficiency (<10 ng/mL severe deficiency, 10-20 ng/mL moderate deficiency) (5).

### Statistical Analysis

The statistical analyses were obtained using the SPSS version 11.5 (Statistical Package for the Social Sciences for Windows) program. Student's t-test or Mann-Whitney U tests were used to compare means between groups, chi-square and Fisher's exact tests were used to compare ratios, and Wilcoxon test was used to evaluate dependent-repeated measurements. Additionally, Pearson correlation analysis was used in the evaluation of two quantitative data. If p value is less than 0.05, it is considered as statistically significant.

## Results

The socio-demographic characteristics of the mothers according to the groups are shown in Table 1.

Of the cases in group I, 45 (43%) were female, 59 (57%) were male, and of the cases in group II, 67 (57%) were female and 51 (43%) were male. Gestational weight of the patients were; in group I, small for gestational age (SGA) (n=18), appropriate for gestational age (AGA) (n=80), large for gestational age (LGA) (n=6) and in group II; SGA (n=11), AGA (n=93), LGA (n=14). 74% of group I cases and 69% of group II cases were delivered by cesarean section. There was no difference between the groups regarding the weight of the patients at birth and gender ( $p>0.05$ ).

The median weight of group I cases was 2390 grams (range 1520-3850 grams), and median height was 45 cm (range 37-50 cm); in group II cases, the median weight was 3410 grams (range 1990-4520 grams), and the median height was 50 cm (range 43-54 cm). There was a significant difference between both groups ( $p<0.01$ ).

**Table 1.** Socio-demographic status of patients' mothers

Parameter	Group I n-%	Group II n-%	p
Maternal age (mean $\pm$ SD years)	28.5 $\pm$ 5.5	28.8 $\pm$ 5.7	0.840
Gestational diabetes (yes)	6-5.7	8-6.7	0.324
Early membrane rupture (yes)	2-1.9	-	0.285
Preeclampsia (yes)	7-6.6	-	<b>0.004</b>
Profession and homemaker	94-90.4	98-83.1	
Other	10-9.6	20-16.9	0.120
<b>Education level**</b>			
Illiterate	2-1.9	5-4.2	
Primary school	77-74	74-64.7	0.292
High school	21-20.2	31-26.3	
University	4-3.8	8-6.8	
<b>Vitamin use in pregnancy</b>			
Yes	73-70.2	78-66.1	
No	31-29.8	40-33.9	0.565
<b>Vitamin use ng/mL in pregnancy</b>			<b>0.001</b>
Yes	8.6 $\pm$ 7.3	8.8 $\pm$ 7.3	
No	5.4 $\pm$ 3.7	4.8 $\pm$ 3.7	
<b>Gravity</b>			
$\leq 3$	80-86.5	103-87.3	
$> 3$	24-13.5	15-12.7	0.613
<b>Parity</b>			
$\leq 2$	72-69.2	87-73.8	0.578
$> 2$	32-30.8	31-26.2	

\*\*Chi-square and Fisher's exact tests, SD: Standard deviation

While umbilical cord serum 25-OH vitamin D levels of babies whose mothers had used a vitamin supplement during pregnancy in group 1 and group 2 were 8.6 $\pm$ 7.3 ng/mL and 8.8 $\pm$ 7.3 ng/mL, these measurements were found to be 5.4 $\pm$ 3.7 ng/mL and 4.8 $\pm$ 3.7 ng/mL for the babies whose mothers had not used a vitamin supplement during pregnancy in group 1 and group 2, respectively. Umbilical cord serum 25-OH vitamin D levels are higher in both groups I and group II in patients whose mothers are under multivitamin supplement ( $p<0.01$ ).

The umbilical cord blood vitamin D level was  $<20$  ng/mL in 94.5% (n=69) of group I infants and 92.3% (n=72) of group II infants whose mothers were using vitamins; it was  $<20$  ng/mL in 100% (n=31) of group I infants and 100% (n=40) of group II infants whose mothers did not use vitamins. Umbilical cord vitamin D level was  $<10$  ng/mL in 83% (n=60) of group I infants and 88% (n=68) of group II infants whose mothers were using vitamins; it was  $<10$  ng/mL in 80% (n=25) of group I infants and 58% (n=23) of group II infants of mothers who did not use vitamins. Although vitamin D usage during pregnancy increased the level of vitamin D, it did not prevent vitamin D deficiency ( $p=0.753$ ).

Table 2 shows the vitamin D levels and the incidence of vitamin D deficiency and insufficiency according to the groups.

Vitamin D levels were re checked at the age of four months in 70 infants in group I and 76 infants in group II. The mean vitamin D level of group I was 34.4  $\pm$  8.7 ng/mL, and the mean vitamin D level of group II was 38.9 $\pm$ 12.7 ng/mL at the age of four months. It was found that vitamin D level was  $<10$  ng/mL in 3.9% and  $<20$  ng/mL in 7% of group I infants. Vitamin D levels were found to be  $<10$  ng/mL in 6.9% and  $<20$  ng/mL in 10.5% of group II infants. There was no statistically significant difference between groups by the means of mean vitamin D level ( $p>0.05$ ) (Table 2).

With the administration of vitamin D in both groups, the vitamin D levels at the 4<sup>th</sup> month were increased statistically significantly compared to the vitamin D level in the umbilical cord blood (Wilcoxon test,  $p=0.02$  for group I and  $p=0.01$  for group II).

In the group I, 46% of cases were fed with breast milk, 50% with breast milk and formula, and 4% with only formula. In group II, 63.2% of cases were fed only breast milk, 31.6% with breast milk and formula, and 5.3% with only formula. The enteral feeding types of both groups were similar ( $p=0.455$ ). Vitamin D levels were also similar at the 4<sup>th</sup> month according to the feeding types (Table 3).

**Table 2.** Serum vitamin D level in umbilical cord blood and fourth postnatal month

Initial	Group I n=104	Group II n=118	p
<b>Vitamin D level (ng/mL); mean ± SD</b>	7.6±6.6*	7.5±6.5**	0.835
Vitamin D level (ng/mL); median (range)	6.1 (0.1-30)	5.1 (0.1-28)	0.790
<b>Deficiency; n (%)</b>	<10 ng/mL	85 (81.7)	0.773
	10-20 ng/mL	15 (14.5)	0.696
	<20 ng/mL	100 (96.2)	0.753
<b>Insufficiency; n (%)</b>	<b>20-32 ng/mL</b>	4 (3.8)	0.751
<b>The fourth month</b>	<b>n=70</b>	<b>n=76</b>	
<b>Vitamin D level (ng/mL); mean ± SD</b>	34.4±8.7*	38.9±12.7**	0.890
Vitamin D level (ng/mL); median (range)	40 (7.2-95)	45 (6-95)	0.740
<b>Deficiency; n (%)</b>	<10 ng/mL	3 (3.8)	0.280
	10-20 ng/mL	2 (3.2)	0.990
	<20 ng/mL	5 (7)	0.439
<b>Insufficiency; n (%)</b>	<b>20-32 ng/mL</b>	4 (5.7)	0.812

Wilcoxon \*p=0.02, \*\*p=0.01, SD: Standard deviation

**Table 3.** Comparison of serum vitamin levels according to the patient's nutritional status

	Group I	Group II	p
Breastfeeding n, median (range) ng/mL	32-38 (16-80)	48-40 (6-75)	p>0.05
Breastfeeding + formula n, median (range) ng/mL	35-40 (7-90)	24-38 (8-70)	

**Table 4.** Comparison of serum vitamin levels according to the patient's birth weight

Vitamin D level	Group I n (%)			Group II n (%)			p
	SGA	AGA	LGA	SGA	AGA	LGA	
<10 ng/mL	15 (83)	66 (82)	4 (66)	8 (72)	74 (79)	9 (64)	0.714
10-20 ng/mL	2 (11)	11 (14)	2 (34)	3 (28)	15 (16)	3 (22)	0.840
20-32 ng/mL	-	2 (3)	-	-	3 (3)	1 (7)	0.910
>32 ng/mL	1 (6)	1 (1)	-	-	1 (1)	1 (7)	0.860

AGA: Appropriate for gestational age, LGA: Large for gestational age, SGA: Small for gestational age

When infants' mothers sun exposure, in infants with and without severe vitamin D deficiency in infants (<10 ng/mL) were compared; although those with <10 ng/mL had less weekly sun exposure (<10 ng/mL 5.7±4.4 h; >10 ng/mL 6.0±3.5 h), there was no statistically significant difference (p=0.658).

The mothers of 65 (63%) infants in group I and 77 (65.2%) infants in group II were wearing hijab. Severe vitamin D deficiency was determined at the sample of umbilical cord blood of 55 (84%) newborns in group I and 61 (80%) newborns in group II.

However, severe vitamin D deficiency was found in the umbilical cord blood samples of 30 (77%) babies in group

I and 30 (73%) babies in group II of mothers who were not wearing hijab. There was no statistically significant difference between the groups in terms of dressing style and severe vitamin D deficiency (p>0.05).

There was no statistically difference between the umbilical cord vitamin D deficiency severity and birth weight in late preterm and term infants (p>0.05) (Table 4).

## Discussion

In this study, umbilical cord and fourth month 25-OH vitamin D levels in late preterm and term infants were compared. In both groups, a high rate of vitamin D

insufficiency and deficiency was detected at the cord serum level. It was observed that with the same amount of vitamin D supplementation given to both late preterm and term cases, vitamin D levels were notably normalized in the 4<sup>th</sup> month. With these features, our study is one of the limited number of studies on this topic in the literature.

Although it varies in different countries, vitamin D deficiency is seen at a rate of 20%-85% during pregnancy in worldwide (6). In studies conducted in Turkey, the prevalence of vitamin D deficiency varies between 67.5%-90% (7,8). However, most rates reported previously included term newborn infants and their mothers.

In our study, vitamin D deficiency was found in 96.2% and severe vitamin D deficiency in 81.7% of late preterm infants. In term infants, vitamin D deficiency was found in 94.2% and severe vitamin D deficiency in 77%. Vitamin D levels were within normal limits in only one infant among both groups. This may be because our hospital generally serves a patient population with low socio-economic status and the study was conducted during winter.

If the vitamin D storage of the mother is sufficient, the baby has sufficient vitamin D levels for about 3 months. Particularly in the third trimester of pregnancy, 25-OH vitamin D is transferred from mother to baby. In a study conducted in India, fifty newborns with low calcium levels were examined and it was observed that 26% (n=13) of them were infants who were only breastfed and did not receive vitamin D supplementation. It was also been observed that the mothers of these babies had low levels of vitamin D (9).

In our study, the frequency of mothers receiving vitamin D supplementation was similar. In both groups, umbilical cord vitamin D levels were significantly higher in babies whose mothers used vitamins. However, the use of vitamin D did not reduce the rate of vitamin D deficiency in either groups. The low vitamin D levels of the mothers despite suppression generally during pregnancy may be related to the small amount of vitamin D in the multivitamin content used in our country (400 IU) and the insufficient intake of vitamin D through food sources. This is because the milk and dairy products in our country are not enriched with vitamin D or it may be related to other factors.

In the northern hemisphere, vitamin D production in the skin begins to decrease starting from autumn in regions north of the Tropic of Cancer (10,11). Studies have found that the vitamin D levels of babies born in summer/autumn periods are higher than those born in winter/spring periods (10,11). Similar to the results in a study by Ustuner et al. (12) in 79 pregnant women in the third trimester who

had their pregnancies mostly during the winter months, vitamin D levels were found to be lower. In another study of 171 cases involving prepubertal girls, it was observed that serum 25-OH D levels did not change and could not reach the values measured in the summer months when vitamin D supplementation was given between October and February (13). In a study conducted in Finland, 2 groups were selected, pregnant and non-pregnant women, and serum 25-OH D levels of pregnant women who had their pregnancies mostly during winter months were found to be lower than those of non-pregnant women. In the summer, no difference was observed between the groups. Based on this result, it has been suggested that mothers should be given vitamin D supplementation in winter (14).

The seasonal effect could not be evaluated because most of the case group were babies born in the winter-spring period, in our study. However, the fact that the vitamin D level was <20 ng/mL in most cases in both groups may indirectly indicate that the vitamin D level is seasonally low in winter.

Conditions such as increasing urbanization, dark skin color, covered clothing, not using the vitamin support given during pregnancy, low socio-economic level, and die poor in vitamin D and calcium may cause changes in maternal vitamin D level.

In a study conducted with breastfeeding mothers and their babies in Asia, the effect of socio-economic level on blood vitamin D levels was investigated. The serum 25-OH D level was found to be significantly lower in mothers with high economic income and their babies compared to mothers with low income and their babies. This has been attributed to the fact that those with high incomes do not go out to the sun as much (15). In a study conducted in our country, it was found that 57% of mothers of infants with rickets were illiterate (16). Contrary to this information, in another study, no correlation was found between serum 25-OH D3 levels in infants and the socio-economic status of the mother (17). In our study, the rate of illiterate mothers was 1.9% in group I and 4.2% in group II. There was no correlation between the education level of the mothers and the serum 25-OH D level of the babies.

Serum 25-OH D3 levels were found to be low in 70% of the mothers wearing covered clothing and their babies in a study conducted in Saudi Arabia (17).

In our study, no significant relationship was found between the level of education and vitamin D level. The mothers of the babies in the groups were similarly dressed in covered clothing. No significant difference was found between clothing style and umbilical cord blood vitamin D levels compared with other studies. This may be due to the

low level of vitamin D in most of the cases and the fact that they were born in the winter months.

The main source of vitamin D in humans is sunlight. Any factor that prevents the sun's rays from reaching the skin reduces the synthesis of vitamin D (1,2,18). Holick (19), in their study, found that the most important factors affecting the synthesis of vitamin D in the skin were the size of the skin area exposed to sunlight and the time interval during the day. In our study, no correlation was found between the duration of sunlight exposure and vitamin D level. Weekly sun exposure times were similar in both groups.

In a study, they could not find any correlation between serum 25-OH D levels, height and weight values of infants (20). Giapros et al. (21) examined 128 late preterm in 102 AGA and 26 SGA types, and reported similarly low vitamin D levels in SGA and AGA infants in the early period (SGA  $20 \pm 7$  ng/mL; AGA  $21 \pm 11$  ng/mL), and normal vitamin D levels at the 6<sup>th</sup> month in both groups ( $45 \pm 14$ ,  $47 \pm 10$  ng/mL). In our study, the results were compatible with the literature.

The optimal level of vitamin D is debatable (22,23,24,25,26,27,28). It has been suggested that the target level of vitamin d should be the level that suppresses PTH, which is published as 40 ng/mL (7). The serum 25-OH D concentration required to prevent osteomalacia is more than 15 ng/mL, while the serum 25-OH D concentration required to improve neuromuscular performance is 38 ng/mL (22). The concentration of serum 25-OH D found to reduce the risk of colon cancer by 50% is 33 ng/mL, and the level of 25-OHD decreasing the risk of breast cancer by 50% is 52 ng/mL (23,24).

In a study, they reported that daily use of 3000 IU vitamin D, which is recommended for pregnant women, brought the serum 25-OH D level to  $>35$  ng/mL in 97% of the cases (25). In infants who are not exposed to sun light, 1000-2000 IU vitamin D support should be given daily by planning according to their body mass. Halicioglu et al. (8) were stated that daily vitamin D needs should be reviewed, especially in infants whose mothers have vitamin D deficiency. However, it was stated that the risk of intoxication might be increased by giving high doses (1,2).

In our study, after 400 IU/day vitamin D was given to all subjects, the repeated blood samples showed that vitamin D level at the 4<sup>th</sup> month was found to be  $>20$  ng/mL in 93.1% of

group I and 89.5% in group II. Vitamin D treatment increased the level of 25-OH vitamin D statistically significantly.

### Study Limitations

The main limitations of this study is that it was conducted in a single center with a limited number of cases. Other shortcomings are that the infants-constituting the study group were born in the winter and the cord blood was collected in the initial period and then the cases were checked in the fourth month.

## Conclusion

As a result, although the cord blood vitamin D levels of late preterm infants were similar to term infants, a high level of vitamin D deficiency was found in the cord blood of both groups. Repeated vitamin D-level control in the fourth month showed that vitamin D levels increased, increased significantly and returned to normal levels with oral 400 IU vitamin D3 replacement. Infants with vitamin D deficiency should be administered vitamin D prophylaxis immediately after birth as soon as feeding is tolerated.

### Ethics

**Ethics Committee Approval:** The study was planned in accordance with the Declaration of Helsinki after obtaining permission from the University of Health Sciences Turkey, Zeynep Kamil Maternity and Children's Training and Research Hospital, Ethical Committee (decision no: 034, date: 15.02.2013).

**Informed Consent:** Parents of the babies were informed about the study and their consent were obtained.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: D.Y.Ö., S.E., Concept: D.Y.Ö., S.E., Design: D.Y.Ö., S.E., G.K., Data Collection or Processing: D.Y.Ö., S.E., A.K., G.K., Analysis or Interpretation: D.Y.Ö., S.E., A.K., G.K., Literature Search: D.Y.Ö., S.E., A.K., G.K., Writing: D.Y.Ö., S.E.

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