



ARAŞTIRMA / RESEARCH

Relationship between umbilical cord blood vitamin D levels and thymus size in healthy and term newborns

Sağlıklı term yenidoğanlarda umbilikal kord kanında D vitamini düzeylerinin timus büyüklüğü ile ilişkisi

Selvi Gülaşi¹, Mustafa Kurthan Mert¹, Gökhan Söker², Ümit Çelik³

¹T.C. University of Health Sciences, Adana City Research and Training Hospital, Department of Pediatrics Neonatology, ²Department of Radiology, ³Department of Pediatrics, Adana, Turkey

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Abstract

Purpose: Thymus is a primary lymphoid organ which contains the vitamin D receptor. Vitamin D has an immunological regulatory effect on the adaptive and innate immune system. The aim of this study was to determine the relationship between cord blood 25-hydroxy vitamin D levels and thymus size.

Materials and Methods: 149 babies were included in the present study. The cord blood 25-hydroxy vitamin D level was measured. Mothers' and infants' features were recorded. Thymus volume were evaluated with ultrasonography. The thymic index and thymus/weight index were calculated.

Results: In the case of irregular use or non-use of maternal vitamin D, the likelihood of vitamin D deficiency in the cord blood was higher. No difference was observed between the thymic index measurements and maternal vitamin D use and cord blood vitamin D level.

Conclusion: There was no relationship between cord blood vitamin D level and thymus size.

Keywords: Newborn, thymus, ultrasonography, vitamin D

Öz

Amaç: Timus vitamin D reseptörü içeren primer bir lenfoid organdır. D vitamini adaptif ve doğal immün sistem üzerine düzenleyici etkilere sahiptir. Bu çalışmanın amacı kord kanı 25-hidroksi vitamin D düzeyi ile timus büyüklüğü arasındaki ilişkiyi tanımlamaktır.

Gereç ve Yöntem: Çalışmaya 149 bebek alındı. Kord kanı 25-hidroksi vitamin D düzeyleri ölçüldü. Annelerin ve bebeklerin özellikleri kaydedildi. Timus büyüklüğü ultrasonografi ile değerlendirildi. Timik indeks ve timus/ağırlık indeksi hesaplandı.

Bulgular: Annenin D vitaminini düzensiz kullanması veya kullanmaması durumunda kord kanında D vitamini eksikliği olasılığını daha yüksekti. Timik indeks ile annenin vitamin kullanımı ve kord kanı D vitamini düzeyi ile timik indeks arasında ilişki saptanmadı.

Sonuç: Kord kanı D vitamini düzeyi ile timik indeks arasında ilişki saptanmamıştır.

Anahtar kelimeler: Yenidoğan, timus, ultrasonografi, vitamin D

INTRODUCTION

Vitamin D is a steroid hormone dissolving in fat. It contributes to the mineralization of the skeleton and calcium homeostasis. Recent studies have indicated that vitamin D is a key modulator of the immune function and inflammation, which have widespread regulatory effects on the adaptive and innate immune system¹⁻⁵. Vitamin D reveals its biological effects

through vitamin D receptor (VDR) in such various organs and tissues as the kidneys, the intestinal mucosa, and immune cells as well as the osteocytes⁶. All tissues of the immune system have been shown to express VDR⁷. It has been demonstrated in the related studies conducted that vitamin D induces the production of antimicrobial cathelicidin, peptides such as beta-2 and beta-3 defensins, that it plays a role in the differentiation of T helper cells (Th1 and Th2), that it is anti-inflammatory for neutrophils and

Yazışma Adresi/Address for Correspondence: Dr. Selvi Gülaşi, T. C. University of Health Sciences, Adana City Research and Training Hospital, Department of Pediatric Neonatology, Adana, Turkey E-mail: selvigulasi@mynet.com
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prevents excess release of cytokine, and that increases the motility of neutrophils, therefore contributing to the regulation of inflammatory response⁸⁻¹².

Thymus is a primary lymphoid organ which provides a suitable micro environment for the differentiation and maturation of T cells, is of utmost importance in the development of the natural and adaptive immune system, and serves as a bridge between immune and neuroendocrine systems¹³⁻¹⁵. The differentiation of T cells is a thoroughly-arranged process. The mature T cells with immunocompetence are separated through the blood and the lymphatic vessels, then migrate into peripheral lymphoid organs such as the spleen and lymph nodes. The highest immunological activity of thymus is in the first six month of life when the number of lymphocyte forming in the thymus is the highest^{16,17}. The size of the thymus gradually gets smaller following the first year of life. This is a physiological involution. Though the capacity of thymus to regenerate the immune system seems to be reduced with age, the remaining tissue possesses the ability to produce new T cells with immunocompetence during adolescence and even in old age. The significance of thymus as an organ with immune and endocrine functions continues throughout life¹⁸. Peripheral lymphocytes, macrophages, and thymus contain the vitamin D receptor⁶. The association between thymus size and malnutrition (fetal malnutrition, zinc deficiency) was reported previously¹⁹⁻²¹. But association between thymus size and vitamin D level has not been reported yet. In the present study, the thymus size which give information about thymus immunological function was compared with cord blood vitamin D level because of thymus contains VDR.

MATERIALS AND METHODS

The study was conducted in Adana Numune Training and Research Hospital between May 2015 and May 2016. The trial was approved by the local Human Research Ethics Committee of Adana Numune Hospital. Written consent was taken from the parents. Non-smokers 149 mothers and their healthy term babies (> 37th gestational week and 2500 grams) were included in the study. Informations which may have effected vitamin D levels such as maternal use of vitamin D, the season of birth and use of mothers' head scarf were recorded. Pregnant women with gestational diabetes, hypertension, preeclampsia and/or eclampsia, urinary tract infection, vaginal secretion, fever, chorioamnionitis, prolonged

membrane rupture and systemic and chronic disease were excluded. Cord blood was removed from the placental part of the umbilical cord during delivery. Hydroxy vitamin D level (25-OHD) was measured (Advia Centaur XPT Immunassay Systems, Siemens Healthineers, Germany). The vitamin D level of >50 nmol/L was defined as sufficiency, 30-50 nmol/L as insufficiency and <30 nmol/L as deficiency²². The features of the mothers who used vitamin D regularly (1200 units everyday), who used it irregularly (1200 units some days, not less than three times a week), and who did not use it, and those of their babies were recorded.

Ultrasonography was performed within the 24 hours following the birth by a single radiologist who did not have any knowledge about the babies' mothers. Thymic size was measured using the method described by Hasselbalch et al.²³. Examining the infant in supine position, the largest transverse diameter of the gland was measured. Perpendicular to the diameter, the largest sagittal area of the largest lobe was measured. The average diameter and average area were multiplied to estimate the "thymic index" (TI). A Sonosite MicroMaxx transportable ultrasound scanner (Sonosite, Bellshill, Scotland) with an 8-5 MHz C11e transducer was used for the examination. The thymic size relative to weight, the "thymus/weight index" (TWT), was calculated as the thymic index divided by infant weight in kilograms²⁴.

Statistical analysis

IBM SPSS Statistics Version 20.0 packaged program was used for the statistical analysis of the data. Categorical measurements were summarized in numbers and percentages, while numerical measurements in mean and standard deviation (in median and minimum-maximum where necessary). Chi-square statistical analysis was utilized for the comparison between the level of categorized vitamin D level (normal, slightly low, moderate low, and severely low) and the measurements of maternal vitamin D use and maternal headscarf use.

In the comparison of the measurements of vitamin D and thymic index depending on maternal headscarf use, T-test was used in the independent group, while for the comparison of vitamin D level and the measurement of thymic index based on the level of categorized vitamin D in the non-vitamin D group (since only two groups (slight and moderate) were left), Mann Whitney U test was made use of. Statistical significance was accepted as 0,05 in all of

the tests. SPSS reference: IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

RESULTS

There was no difference between the demographic features of the babies and their mothers who did not use, who irregularly used, and those who regularly used vitamin D (Table I). Vitamin D level was lower in the cord blood of the groups who used vitamin D irregularly or those who did not use it ($p=0,027$). No significant relationship was observed in the TI, TWI and vitamin D levels in any of the groups (Table II).

Thymic index and TWI measurements were found to be similar between the groups whose vitamin D levels were slightly, moderately, and severely low and those whose vitamin D levels were normal.

The correlation between the factors likely to have an effect on the results, such as maternal vitamin D use, religious headscarf use, the baby's birth season, and TI, TWI, and cord blood vitamin D level was tested through ANOVA test and no correlation was identified. The relationship between the factors effect the cord blood vitamin D level was tested through MANOVA multivariate analysis and no relationship was found.

Table 1. Features of the Infants and Mothers

	Maternal vitamin D use			P
	Did not use (n=22)	Irregular use (n=48)	Regular use (n=79)	
Gender, n (%)				
Male	16 (73%)	30 (62%)	45 (57%)	0.395
Female	6 (27%)	18 (38%)	34 (43%)	
Mode of delivery, (%)				
Caesarian section	22 (100%)	46 (96%)	78 (99%)	0.365
Vaginal delivery	0 (0%)	2 (4%)	1 (1%)	
Maternal headscarf use, n (%)				
Did not use	8 (36%)	16 (33%)	31 (39%)	0.798
Use	14 (64%)	32 (67%)	48 (61%)	
Gestational age, (week) (Mean \pm SD)	39.1 \pm 0.4	38.8 \pm 0.7	38.8 \pm 0.6	0.179
Birth weight (kg) (Mean \pm SD)	3364.5 \pm 432.3	3346.7 \pm 398.8	3363.4 \pm 405.4	0.972
Birth height, (cm) (Mean \pm SD)	50.1 \pm 1.1	50 \pm 1.3	50.1 \pm 1.3	0.799
Head circumference, (cm) (Mean \pm SD)	35.3 \pm 0.9	35.1 \pm 1.2	35.1 \pm 1.3	0.625
Upper arm circumference, (cm) (Mean \pm SD)	10.4 \pm 0.8	10.7 \pm 1.1	10.8 \pm 1.3	0.338

Table 2. Thymic index measurements and 25-OHD level in cord blood according to maternal vitamin D use

	Maternal vitamin D use			P
	Did not use n (%)	Irregular use n (%)	Regular use n (%)	
<30 nmol/L	40 (83.3)	52 (65.8)	18 (81.8)	
30-50 nmol/L	8 (16.7)	16 (20.3)	4 (18.2)	
>50 nmol/L	0 (0.0)	11 (13.9)	0 (0.0)	
Cord blood 25-OHD level (nmol/L) (Mean \pm SD)	9.8 \pm 3.05	8.74 \pm 3.36	11.45 \pm 6.91	0.027
Thymic index (cm ³) Mean \pm SD	6.57 \pm 2.79	6.51 \pm 3.83	6.80 \pm 3.36	0.889
TWI (Mean \pm SD)	2.0 \pm 0.89	1.96 \pm 1.14	2.03 \pm 1.0	0.937

DISCUSSION

Vitamin D is a steroid hormone and it has significant immune regulatory and anti-inflammatory effects as well as its key role in mineral homeostasis. Vitamin D receptor is found in various organs and cells such as keratinocytes, pancreatic islet cells, neutrophils, macrophages, dendritic cells, lymphocytes, promyelocytic, monocytes, and thymus^{25,26}. Vitamin D performs most of its biological functions by binding to its receptor found in the cell nucleus and enabling the regulation of gene expression, particularly in B cells and T cells, dendritic cells, neutrophils, and macrophages. It has been reported that one of the possible mechanisms that vitamin D reduces the expression of TLR-2 and TLR-4 protein, that active vitamin D suppresses NF- κ B and thus reduces the transcription of cytokines such as tumor necrosis factor- α , interleukin-6 and interleukin-1 β . It has been suggested that vitamin D activation increases intracellular glutathione levels which reduce excessive production of reactive oxygen species²⁷. It was reported through the studies conducted by Çetinkaya et al.^{28,29} that maternal and neonatal low 25-OHD level was linked to necrotizing enterocolitis in premature babies and that there was an increased risk of the development of early neonatal sepsis in term infants with low vitamin D levels.

The fetus meets its calcium needs through the placenta from the mother and through breastfeeding after the birth. For this reason, vitamin D levels in mothers must be adequate during pregnancy and the lactation period. The 400 IU D vitamin D administered in serious vitamin D deficiency cannot prevent the development of neonatal hypocalcemia. In Turkey, beginning from the 12th gestational week through the six months following the birth, a regular diet, and 1200 IU D vitamin intake as well as exposure to sunlight are recommended by the Ministry of Health³⁰. It was found in the present study that vitamin D level in cord blood was low in the cases in which the mothers used vitamin D irregularly or did not use it ($p=0.024$).

Thymus is an organ which enables T cell differentiation and maturation and which possesses significant immunological functions. The size of thymus and its function are related to each other³¹. A small thymus contains fewer epithelial cells which release thymic hormones and cannot provide the micro environment essential for T cell maturation and differentiation³². The small size of thymus has

been suggested to cause immunological dysfunction which is one of the reasons for low birth weight infants to be prone to infection-related diseases^{19,20}. In a study which demonstrated the relationship between small thymus size at birth, particularly in infants with low birth weight and low zinc level in cord blood, the effect of fetal malnutrition on the size of thymus was emphasized²¹. Moreover, it was shown that the thymic size at birth was associated with mortality in the first year of life. A small thymus at six months seems to be a strong risk factor for mortality due to infectious diseases³³. These findings suggest that the thymic size at birth and at an early age may be an important predictor of immunocompetence.

Thymus sonography is the most non-invasive and most commonly used method in immunologic studies for detecting pathologic conditions associated with the size of thymus^{34,35}. In the present study, it was determined that mothers' non-use or irregular use of vitamin D during pregnancy increased the likelihood of vitamin D deficiency in the cord blood at a statistically significant level. There was no statistically significant difference between the thymic index, TWI and use of vitamin D. In an animal study conducted by Yurdakök et al.³⁶, thymus weight, thymus/weight ratio and histological findings were found to be similar when the vitamin-D deficient rats were compared with the control animals. They concluded that vitamin D is not required for the development of rat thymus.

As it is known that the degree of exposure to sunlight and the maternal vitamin D status may change, the present study compared the 25-OHD levels and TI, TWI measurements between mothers who use or do not use religious headscarf and no significant relationship was found. It was observed that although the seasons of birth were different, the cord blood 25-OHD levels of the infants whose mothers do not use headscarf were similar, the cord 25-OHD level of the infants born in spring whose mothers use headscarf were significantly lower.

The present study has some limitations. First, there are many other factors that have an effect on thymus size and in the present study, no data were available except for the type of dressing, mothers' nutrition status and maternal weight gain in pregnancy. Second, our sample size is small. Third, it is doubtful whether thymus size in healthy term infants reflects the differentiation and maturation of T cells, and therefore, it is recommended that T cell functions of peripheral blood be evaluated.

In conclusion, it is determined that when maternal vitamin D use becomes more regular during pregnancy, vitamin D deficiency level becomes lighter. There is an urgent need to renew efforts at policy and implementation levels about use of vitamin D in pregnancy. There is no relationship between cord blood 25-OHD level and thymic index. Future studies need to be performed in larger groups with T lymphocyte cell measurements to evaluate the association between thymus function and vitamin D level.

Yazar Katkıları: Çalışma konsepti/Tasarımı: GÖ; Veri toplama: GÖ; Veri analizi ve yorumlama: GÖ; Yazı taslağı: GÖ; İçeriğin eleştirel incelenmesi: GÖ; Son onay ve sorumluluk: GÖ; Teknik ve malzeme desteği: GÖ; Süpervizyon: GÖ; Fon sağlama (mevcut ise): yok.

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