ARAŞTIRMA MAKALESİ/ ORIGINAL ARTICLE

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Anne ve Term Bebeklerin Vitamin D ve Kalsiyum Metabolizmasının Değerlendirilmesi ve Bebeklerin Kemik Gücünün Kantitatif Ultrason ile Tayini

Determination of Vitamin D Metabolism and Bone Strength by Quantitative Ultrasound in Term Newborns

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ÖZ

GİRİŞ ve AMAÇ: *Yetersiz D vitamin alımı kemik mineralizasyonunu ve döngüsünü olumsuz etkilemektedir. Gebelikte annenin kemik döngüsü üzerindeki değişiklikler fetusun kemik mineral içeriğinde değişikliğe neden olmaktadır. Bu çalışmada fetal kemik minerilizasyonu üzerinde belirleyici olan D vitamin metabolizmasını etkileyen faktörler, biyokimyasal kemik göstergeleri ve yenidoğanlarda kantitatif ultrason ile değerlendirilen kemik yapısı arasındaki ilişkinin saptanması amaçlanmıştır.*

YÖNTEM ve GEREÇLER: *Celal Bayar Üniversitesi Tıp Fakültesi Hastanesinde doğan 90 term bebek ve annesi çalışmaya alındı. Serum kalsiyum, fosfor, alkalen fosfataz, kemik alkalen fosfataz, 1,25(OH)2D, 25(OH)D, paratiroid hormone ve osteokalsin düzeyleri ölçüldü. Yaşamın ilk 96 saati içinde kantitatif ultrason cihazı ile tibial ölçümleri yapıldı.*

BULGULAR: *Annelerin ortalama 25(OH)D ve 1,25(OH)2D düzeyleri normal sınırlarda saptandı. Yenidoğanlarda ölçülen ortalama Ca düzeyleri ortalama 10.1 ±0.6 mg/dl, P düzeyleri 6.2±1 mg/dl saptandı. D vitamini desteği almayan annelerde serum 25 (OH)D değerleri yüksek (p = 0.02), D vitamini desteği almayan annelerde PTH değerleri yüksek ve osteokalsin değerleri düşük bulundu (sırasıyla P = 0.02 ve p = 0.03). Anneleri D vitamini desteği almayan yenidoğanlarda PTH değerleri daha düşüktü (P= 0.004). Ca takviyesi yapılan annelerde 25 (OH) D değeri yüksekti (P = 0.009). Yenidoğanların SOS değerleri 3127 ± 107 (2900-3389), ortalama Z skoru 0.3 ± 0.7 (-1.4 - 2.6) idi.*

TARTIŞMA ve SONUÇ: *Yenidoğanların SOS skorları ile Ca ve ALP değerleri arasında anlamlı korelasyon gözlendi. Kemik yapısının kantitatif ultrason ile değerlendirilmesi, invaziv olmayan ve pratik bir yöntemdir.*

Anahtar Kelimeler: *Term yenidoğan, Vitamin D, kantitatif ultrason*

ABSTRACT

INTRODUCTION: *Insufficient intake of vitamin D negatively effects bone mineralization and turnover. Changes in mothers' bone turnover during pregnancy effects bone mineral composition of the fetus. The aim of this study was to evaluate the factors, biochemical markers and bone structure assessed by quantitative ultrasonography effecting vitamin D metabolism.*

METHODS: *Ninety term newborn and their mothers included in the study. Serum levels of Ca, P, alkaline phosphatase (ALP), bone ALP (BALP), 1,25(OH)2D, 25(OH)D, parathyroid hormone (PTH), and osteocalcine levels were analyzed from the serums of mothers. Quantitative ultrasound from the tibial bone was performed for all infants during the first 96 hours*

RESULTS: *Mean values for 25(OH)D and 1,25(OH)2D were within normal ranges in mothers. Mean blood Ca levels of the newborns was 10.1 ±0.6 mg/dl, and mean P levels of the newborns was 6.2±1 mg/dl. Serum 25(OH)D values were high in mother who used vitamin D supplementation (P=0.02), PTH values were high and osteocalcine values were low in mothers who did not used vitamin D supplementation (P=0.02 and P=0.03, respectively). PTH values were low in newborns whose mothers did not received vitamin D supplementation (P=0.004). 25(OH)D values were high in mothers who received Ca supplementation (P=0.009). SOS values of newborns were 3127±107 (range, 2900-3389), and mean Z scores were 0.3±0.7 (range, -1.4 - 2.6).*

DISCUSSION AND CONCLUSION: *Significant correlation was observed between the SOS scores and Ca and ALP values of the newborns. Assessment of the structure of bone with quantitative ultrasound is a noninvasive and practical method.*

Keywords: *Term newborn, D vitamin, quantitative ultrasound*

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INTRODUCTION

Vitamin D is a steroid hormone that is essential for calcium (Ca) homeostasis and maintenance of bone health (1). Vitamin D deficiency may lead to decrease in serum Ca, resulting in an increase in bone turnover and a decrease in bone mineral accretion (2). Vitamin D supplementation during pregnancy improves neonatal handling of Ca (3,4).

The fetus is dependent on mineral status and the supply through the placenta for both Ca and vitamin D. Vitamin D is mainly transported through the placenta as 25(OH)D (5). The placenta synthesizes 1-25(OH)2D from 25(OH)D for both the mother and the fetus (6). Poor maternal vitamin D status may adversely affect fetal growth, bone ossification and neonatal Ca homeostasis (7).

Factors such as; seasons of birth, intrauterine growth retardation, insulin dependent diabetes, smoking, excessive caffeine intake, and calcium supplementation affects in utero mineral accretion (8). The possible mechanisms associated with these alterations in newborn bone mineralization are unclear. Since effects on fetal bone are often mediated through the mother, any disturbance in maternal mineral metabolism, such as nutrient deficiency or disease, or conditions affecting placental mineral transfer may decrease bone mineralization (8).

Quantitative ultrasound (QUS) measurement of tibial speed of sound (SOS) has been used for the diagnosis of osteoporosis. It is an inexpensive, portable method and free from ionizing radiation (9). Previous reports demonstrated that QUS can successfully assess bone strength in neonates (10).

The aim of this study was to evaluate the factors that influence vitamin D metabolism and fetal bone mineralization including biochemical markers, and to assess bone structure by QUS in term neonates.

MATERIALS AND METHODS

Ninety term newborns which were born in Celal Bayar University Hospital and their mothers were enrolled in this study. The protocol for this study was approved by the ethical committee of Celal Bayar University School of Medicine. Informed consent was obtained from the mothers of all of the neonates who were enrolled in the study.

Serum levels of Ca, phosphorus (P), alkaline phosphatase (ALP), bone ALP, 1,25(OH)2D, 25(OH)D, parathyroid hormone (PTH), and osteocalcine were analyzed from the serums of the mothers (during the first four postpartum days) and the newborns (between the fourth and the tenth days). Lawson Wilkins Pediatric Endocrine Society Drug and Therapeutics Committee defined 25(OH)D level 15-20 ng/ml as vitamin D insufficiency and <5 ng/mL as severe vitamin D deficiency (11).

QUS was performed for all of the infants during the first four days. Speed of sound (SOS) was determined on the newborn infant's leg using Omnisense 7000P (Sunlight Ultrasound Technologies, Rehovot, Israel) ultrasound and cortex small (cs) probe, which is suitable for use on the newborn infant's leg. After applying ultrasound gel to the leg and to the probe a SOS measurement is obtained on the midshaft tibia. All QUS measurements were made by the same investigator (EC). Results were noted as SOS and Z score. Z score is the difference between SOS results of the patient and the mean SOS of the population at the same age and sex and it is defined as standard deviation.

Gestational age, birth weight, birth height, parity of the mothers, weight gain during pregnancy, smoking, alcohol and caffeine intake during pregnancy, presence of vitamin supplementation was recorded. Daily caffeine intake of 300 mg or over was noted as caffeine consumption.

Infants with intrauterine growth retardation, severe central nervous system disease, neurological abnormality, bone or muscle disease, congenital malformation, and, mothers with maternal malabsorption, preeclampsia, renal failure, or diabetes and mothers who used long term phosphate enema were excluded from the study.

Infantile and maternal blood samples were centrifuged, and serums were separated. The samples were stored at -80 ºC. Ca, P, and ALP levels were measured with spectrophotometric method in DXC 800 auto analyzer using Beckman Coulter Galway, Irish kits, bone ALP level with ELISA method (OCTEIA Ostase BAP Immunodiagnostics Systems LTd.Tyne&Wear, UK kits), vitamin 1,25(OH)2D, and vitamin 25(OH)D with ELISA method (Immunodiagnostics Systems LTd. Tyne&Wear, UK kits), parathyroid hormone (PTH) and osteocalcine levels with immulite 2000 autoanalyzer (BIODPC, Los Angeles, USA) kits at Celal Bayar University Biochemistry laboratory.

Statistical Analysis

Qualitative data are presented as counts and percentages. Quantitative data was given as mean ± standard deviation for normally distributed data. A computer software SPSS (Statistical Package for the Social Science) was used for the statistical analysis. Students' t-test was used to compare the differences between the patient and control groups of the normally distributed data. Pearson's correlation analysis is used to measure of the strength of the association between the two numeric variables. P values lower than 0.05 were considered statistically significant.

RESULTS

Mean age of the mothers were 25.5±3.9 years. Characteristics of the mothers are summarized in **Table** 1 and bone biochemical markers and vitamin D levels of them are summarized in **Table** 2.

Table 1. Characteristics of mothers

	n (%)
Parity (primipara/multipara)	**47/43 (52/48)**
Delivery status (vaginal/C/S)	**35/55 (39/61)**
Weight gain during pregnancy (kg)	**12.2±3.9**
Maternal smoking	**21 (23)**
Caffeine intake	**65 (72)**
Smoking and/or caffeine intake	**68 (75)**
Vitamin D supplementation (500 U/day)	**46 (51)**
Calcium supplementation (1500 mg/day)	**35 (39)**

SD, standard deviation; C/S, cesarean section.

Table 2. Bone biochemical markers and vitamin D levels of mothers

Mothers	Mean ± SD
Ca (mg/dl)	9.1±0.5
P (mg/dl)	4.4±0.8
ALP (U/l)	111.8±34.9
Bone ALP (U/l)	18.4±18.3
PTH (pg/ml)	44.6±31.3
25(OH)D (nmol/l)	218.5±74.9
1,25(OH)$_2$D (nmol/l)	85.3±34.8
Osteocalcine (pg/ml)	32.9±17.9

Of the 90 newborns 49 (54%) were female and 41 (46%) were male. Mean gestational age was 39.4±0.8 weeks, mean birth weight was 3251±450 g, and mean birth height was 49.3±1.3 cm.

Bone biochemical markers and vitamin D levels of newborns are summarized in **Table** 3.

Table 3. Bone biochemical markers and vitamin D levels of newborns

Newborns	Mean ± SD
Ca (mg/dl)	**10.1±0.6**
P (mg/dl)	**6.2±1.0**
ALP (U/l)	**143.4±45.4**
Bone ALP (U/L)	**55.9±24.9**
PTH (pg/ml)	**50.3±50.9**
25(OH)D (nmol/l)	**59.8±40.3**
1,25(OH)$_2$D (nmol/l)	**154.4±107.6**
Osteocalcine (pg/ml)	**76.8±30.5**

Mean values for 25(OH)D and 1,25(OH)2D were within normal ranges in all of the mothers. Significant negative correlations were observed between the mothers' PTH and newborns' 25(OH)D levels, and mothers' 25(OH)D and newborns' PTH levels (p=0.006, R=-0.341, and p=0.007, R=-0.314; respectively). 25(OH)D levels were low in 10 (11%) newborns. 1,25(OH)2D levels of the newborns were within normal range. We further grouped newborns as low 25(OH)D levels (Group 1) and normal 25 (OH)D levels (Group 2). 1,25(OH)D, 25(OH)D, and Ca levels were lower in mothers of the newborns in Group 1 compared to those in Group 2 (p=0.039, p=0.035, p=0,042, respectively); no significant difference was observed for SOS and Z scores (**Table** 4).

Table 4. Comparison of the biochemical markers and SOS and Z scores of the group: Newborns having low 25(OH)D levels (Group 1) and newborns having normal 25 (OH)D levels (Group 2)

	Group 1	Group 2	*P*
Ca (mg/dl)	8.8±0.4	9.2±0.4	**0.039**
P (mg/dl)	4.5±0.7	4.3±0.7	**0.608**
ALP (U/l)	127.0±29.8	108.6±36.0	**0.106**
Bone ALP (U/l)	14.8±5.9	18.9±19.4	**0.194**
PTH (pg/ml)	64.2±51.8	40.2±25.2	**0.182**
25(OH)D (nmol/l)	58.2±41.6	91.6±32.2	**0.035**
1,25(OH)$_2$D (nmol/l)	179.6±61.4	227.8±72.4	**0.042**
Osteocalcine (pg/ml)	28.8±10.0	32.6±18.7	**0.362**
SOS (m/sec)	3141.6±113.8	3126.4±101.0	**0.698**
Z score	**0.4±0.9**	**0.3±0.7**	**0.896**

Mean SOS value was 3127±107 (2900-3389) m/sec, and mean Z score was 0.3±0.7 (-1,4-2,6). There was no significant correlation between SOS, Z score and newborns gender and birth weight. Significant correlation was observed between the SOS scores and Ca (positive), ALP (negative) values of the newborns (Figure 1-2).

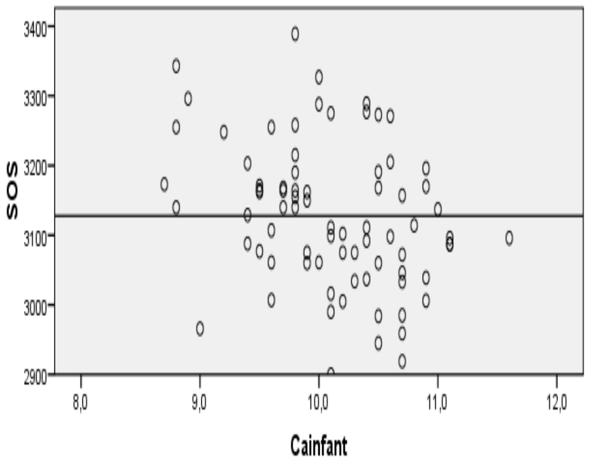

Figure 1. Correlation between newborns' SOS scores and Ca levels

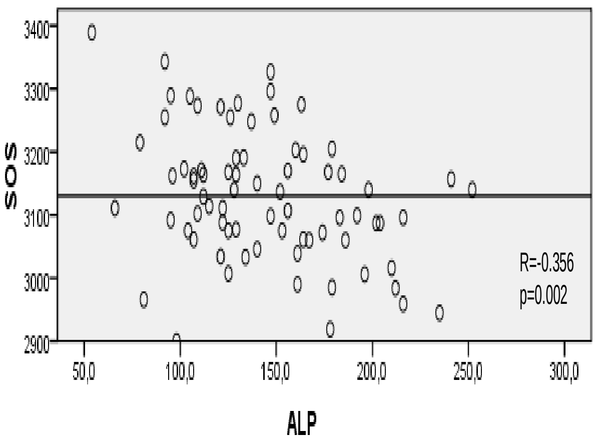

Figure 2. Correlation between newborns' SOS scores and ALP levels.

There was not any significant correlation between parity of the mothers and bone biochemical markers of neither the newborns and nor the mothers. There was not any correlation between parity of the mothers and SOS and Z scores of newborns, either. Mean weight gain of mothers during pregnancy was 12.2±3.9 kg and there was no correlation between weight gain during pregnancy and SOS scores, or bone biochemical markers of the mothers and the newborns (p>0.05).

SOS scores of newborns whose mothers consumed caffeine during pregnancy were lower than the newborns whose mothers did not consume caffeine products (3088±85 m/sec and 3187±112 m/sec; respectively) (p=0.022). No significant effect of smoking was observed on the mothers' or newborns' bone biochemical markers or SOS scores (p>0.05).

Serum 25(OH)D and Ca levels were high in the mothers who received vitamin D supplementation (p=0.042 and p=0.03; respectively), PTH levels were high and osteocalcine levels were low in the mothers who did not use vitamin D supplementation (p=0.02 and p=0.03; respectively). PTH levels were low in the newborns whose mothers did not receive vitamin D supplementation (p=0.004).

No significant correlation was observed between vitamin D supplementation of the mothers during pregnancy and SOS or Z scores of newborns (p>0.05). No significant correlation was observed between Ca supplementation of the mothers during pregnancy and SOS or Z scores of the newborns (p>0.05). 25(OH)D levels of the mothers who received Ca supplementation during pregnancy were higher compared to the mothers who did not receive Ca supplementation (78.8±31.5 and 100.0±32.3, respectively) (p=0.009).

DISCUSSION

Basically, effects on the fetal bone originate from the mineral status of the mother. There is a positive correlation between umbilical cord and maternal blood 25(OH)D levels but the level in the cord blood is lower than the maternal level (12). Similarly, in this study we observed a positive correlation between 25(OH)D levels of the mothers and the newborns and mothers' 25(OH)D levels were higher than those of the newborns.

The lowest limit of serum 25(OH)D level is between 20 nmol/l (8,3 ng/ml) and 80 nmol/l (33 ng/ml). For although there is not any significant cut-off values for 25(OH)D levels, values lower than 20 nmol/L form the limit for hypovitaminosis (11). In this study none of the mothers had 25(OH)D levels lower than 20 nmol/l.

Pawley et al., in their study that they compared mothers taking 1000 U/day vitamin D with controls, observed that cord blood 25(OH)D levels were significantly lower in the control group and that there was not any difference between ionized Ca, Mg, and PTH levels in the groups. Birth weight was higher in the vitamin D and Ca treatment group (13). In this study serum 25(OH)D, and Ca levels were higher in the mothers who received 500 U/day vitamin D supplementation. PTH levels were higher and osteocalcine levels were lower in the mothers who did not use vitamin D supplementation. PTH levels were low in the newborns whose mothers did not receive vitamin D supplementation. No significant correlation was observed between vitamin D supplementation and birth weight, serum ALP, or P levels of the newborns.

Devlin et al., in their study, pointed out that mother's serum Ca, PTH or 1,25(OH)2D levels in the vitamin D treatment group after the 6th month did not differ significantly, but the increase in 25(OH)D levels was significant. 25(OH)D levels were lower and 1,25(OH)2D levels were higher in the infants in the control group. There was not any difference between Ca, Mg and PTH levels of the infants (14).

In the study with 1139 Scottish mothers by Cockburn et al., one group took 400 IU vitamin D starting on the 12th week and one group did not. 25(OH)D and Ca levels were higher in the vitamin D group on 24th and 34th week in maternal blood, cord blood, and 6 days old infants' blood samples when compared with the control group (15). In another double blind, randomized study in which 126 mothers received 1000 U/day vitamin D or placebo, maternal Ca and plasma Ca levels of infants on the 3rd and 6th days were higher in the vitamin D group (16).

Children of the mothers who smoked during pregnancy showed weight, height and bone (lumbar) density retardation in prepubertal period (17,18). Gonnelli et al., studied quantitative ultrasound scale parameters in term and preterm infants and could not find any statistically significant correlation between these parameters and maternal smoking, Ca treatment, or familial osteoporosis history. This result was thought to be due to the small number of smoking mothers (19). Similarly, we did not find a correlation between SOS values and maternal smoking, because the number of smoking mothers and the number of cigarettes they smoked were low.

Maternal caffeine intake results in reduced fetal weight, and bone Ca, and Mg content in rats (20). In this study SOS scores of the newborns whose mothers consumed caffeine during pregnancy were lower than the newborns whose mothers did not consume caffeine products.

There are some studies relating low cord blood Ca level with childhood low bone density. Placental capacity leading to materno-fetal Ca passage affects postnatal skeletal development21.Also in this study significant correlation was observed between the SOS scores and Ca (positive), ALP (negative) values of the newborns and all results were within normal range.

25(OH)D levels were low in 10 newborns. 1,25(OH)2D levels of newborns were within normal range. 1,25(OH)D, 25(OH)D, and Ca levels were lower in mothers of the newborns whose 25(OH)D levels were lower compared to the normal ones. No significant difference was observed in SOS and Z scores. Also, the mothers of the neonates with low 25(OH)D vitamin levels did not have hypovitaminosis. When the number of the factors affecting bone development was taken into account, it was not unexpected to find normal SOS and Z scores in neonates with low 25(OH)D levels.

Effects of using minerals during pregnancy on the maternal and fetal mineral changes have been unclear. Healthy women transmit sufficient minerals to the fetus without mineral support (21). Fetal humeral ossification was retarded in animals which follow an insufficient Ca diet for 2 months in the second trimester (22). Although 25(OH)D levels were lower in the mothers who were not taking Ca when compared with the Ca treatment group, it was still within normal range and there was not vitamin D insufficiency in their infants because of this it was thought that SOS scores of quantitative ultrasound were in normal range. In addition to Ca replacement, taking sufficient Ca with diet is also important.

Nemet et al., studied on 20 neonates and observed that bone SOS values were lower in the neonates with extremely low birth weight (ELBW) when compared with the neonates with low birth weight (LBW) and very low birth weight (VLBW) (23). Littner et al., measured SOS values in 73 term and preterm infants and found significant relation between SOS values and gestational age, and birth weight. No statistically significant difference found for gender (24). Yiallourides et al., could not find a correlation between SOS values and birth weights of term and preterm infants (25). Tansug et al., studied on 78 preterm and 48 term infants and they observed significant difference on 10th day measurements of SOS but there was no difference between 12th month values between two groups (26). In this study there was no correlation between SOS scores of newborns and gestational age, or birth weight. We thought that this result may be due to the newborns who were enrolled in the study were term, birth weights were similar and newborns small for gestational age were not enrolled the study.

Nemet et al., found a negative correlation between the tibial SOS values and serum ALP. In their study, infants with high ALP and low phosphate levels had the lowest SOS values (23). In our study there was a positive relation between SOS values and neonatal Ca levels and a negative relation between the SOS values and ALP levels.

Quantitative ultrasound method is being used more frequently to evaluate bone strength because it is easy to perform, portable, cheap and non-radioactive. Average SOS values of some studies with term neonates were as follows; 3079 m/sec, in term neonates younger than 8 days (McDevitt et al.), 3100 m/sec in term neonates younger than 3 days (McDevitt et al.), 2850-3300 m/sec in 25 twin neonates (Rauch & Schoeanau), 2850-3300 m/sec in 73 term neonates younger than 5 days (Littner et al.), 3101 m/sec in 44 term neonates younger than 3 days (Nemet et al.) (27). Results are similar in our study and average SOS value was 3127±107 m/sec (range: 2900-3389).

Kara et al. evaluated of bone metabolism in newborn twins using quantitative ultrasound and found that the phosphorus level and the maternal drug use were significantly reduced SOS (28). In our study there was not a relation between SOS values and P levels.

Sufficient vitamin supplementation and nutrition of the mothers during pregnancy effects bone mineralization of the newborn. Assessment of the structure of bone with quantitative ultrasound is a noninvasive, practical, cheap and reliable method. Beside bone biochemical markers and hormone levels, quantitative ultrasound can be used to evaluate the bone strength of the newborns.

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