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Research Article / Araştırma Makalesi

# 25-Hydroxyvitamin D Levels in Preterm Infants ≤32 Weeks Gestational Age and Risk of Late Onset Neonatal Sepsis

## *Gebelik Yaşı ≤32 Hafta Olan Prematüre Bebeklerde 25-Hidroksivitamin D Düzeyleri ve Geç Başlangıçlı Sepsis Riski*

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## Abstract

The aim of this study was to evaluate neonatal vitamin D status and effect of vitamin D levels on the development of late-onset sepsis (LOS) in preterm infants with a gestational age of ≤32 weeks. Newborns having a gestational age of ≤32 weeks with culture proven LOS consisted the study group, whereas the control group consisted of gestational age matched newborns hospitalized in the neonatal critical care unit with no evidence of clinical or laboratory infection. 58 (47.5%) had culture proven LOS (study group), while 64 (52.5%) had no signs or symptoms of sepsis (control group). Median 25-hydroxyvitamin D (25-OHD) levels of study group was significantly lower than the median 25-OHD levels of the control group (10.2 ng/ml vs 18.3 ng/ml; p=0.0001). Statistically significant higher rates of low vitamin D levels (25-OHD level <15 ng/ml) were observed in the study group compared to control group (50/58, 86% vs 23/64, 36%; p=0.0001). Preterm infants with low 25-OHD levels were 15.2 (95% confidence interval (CI):5.14-45.10; p=0.0001) times more likely to experience LOS compared with the preterm infants with normal 25-OHD levels. Up to now, there is no established optimal 25-OHD level for adequate immune function for preventing neonatal sepsis in both term and preterm infants, but in this study preterm infants with LOS were found to have significantly lower 25-OHD levels compared to preterm infants at the same gestational age without LOS and low 25-OHD levels seem to increase risk of neonatal LOS.
**Keywords:** 25-hydroxyvitamin D, preterm infant, season, late-onset sepsis

## Özet

Bu çalışmanın amacı, gebelik yaşı ≤32 hafta olan prematüre bebeklerde D vitamini düzeyinin geç başlangıçlı sepsis gelişimine etkisini değerlendirmektir. Çalışma grubunu gestasyon yaşı ≤32 hafta olan ve kültür ile kanıtlanmış geç başlangıçlı sepsis saptanan yenidoğanlar oluştururken, yenidoğan yoğun bakım ünitesinde yatan, ≤32 gebelik haftası olan ve klinik veya laboratuvar enfeksiyon bulgusu olmayan yenidoğanlar kontrol grubunu oluşturmaktadır. 58'inde (%47,5) kültürle kanıtlanmış geç başlangıçlı sepsis (çalışma grubu) varken, 64'ünde (%52,5) sepsis belirti veya semptomu yoktu (kontrol grubu). Çalışma grubunun ortanca 25-hidroksivitamin D (25-OHD) seviyeleri, kontrol grubunun ortanca 25-OHD seviyelerinden anlamlı derecede düşüktü (10,2 ng/ml'ye karşın 18,3 ng/ml; p=0,0001). D vitamini düzeyi düşük bebeklerin oranı çalışma grubunda kontrol grubuna kıyasla istatistiksel anlamlı olarak daha yüksek (25-OHD düzeyi <15 ng/ml) bulundu (50/58, %86'ya karşın 23/64, %36; p=0,0001). 25-OHD düzeyi düşük olan prematüre bebeklerin, normal 25-OHD düzeyine sahip prematüre bebeklere kıyasla geç başlangıçlı sepsis yaşama olasılığı15.2 (%95 güven aralığı (GA):5,14-45,10; p=0,0001) kat daha fazlaydı. Prematüre ve term yenidoğanlarda neonatal sepsisin önlenmesi için yeterli bağışıklık fonksiyonu için belirlenmiş bir optimal 25-OHD seviyesi yoktur ancak bu çalışmada geç başlangıçlı sepsisi olan prematüre bebeklerin, erken başlangıçlı sepsisi olmayan prematüre bebeklere kıyasla önemli ölçüde daha düşük 25-OHD düzeylerine sahip olduğu ve düşük 25-OHD seviyelerinin yenidoğanın geç başlangıçlı sepsis riskini arttırdığı bulunmuştur.
**Anahtar Kelimeler**: 25-hidroksivitamin D, preterm bebek, mevsim, geç başlangıçlı sepsis

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## 1. Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs and symptoms of an infection with a specific agent grown in blood culture in the first month of life. It is also a major cause of morbidity and mortality in preterm infants. The incidence of neonatal sepsis ranges from one to eight neonates per 1000 live births (1). According to the onset of clinical findings of sepsis, neonatal sepsis could be classified as early-onset (EOS), late-onset (LOS), or very late-onset sepsis (2).

Vitamin D has a key role in immune function. Recent research has suggested that vitamin D plays a role in promoting the normal function of the innate and adaptive immune systems (3). Vitamin D is a lipid-soluble steroid hormone best known for its role in calcium homeostasis and bone health in both children and adults (4). However, it has been the subject with great interest because of its "non-classical" actions in tissues unrelated to calcium homeostasis, particularly in the regulation of both innate and adaptive immune system (5). In addition, vitamin D supplementation has been shown to reduce infections in children and to aid in the prevention of autoimmune disorders (6).

The association of vitamin D deficiency and sepsis has been reported in neonates; however, there is limited data on the association of LOS and vitamin D deficiency in preterm infants (5,7).The aim of this study was to evaluate neonatal vitamin D status and effect of vitamin D levels on the development of LOS in preterm infants with a gestational age of ≤32 weeks. The association between LOS and severity of vitamin D deficiency was secondary outcome of this study.

## 2. Materials and Methods

This single center retrospective cohort study was conducted between April 2019 and April 2021 at Dörtcelik Children's Hospital. The research has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee. Parental informed consent was obtained from each patient included in the study.

Our center does not have an obstetrics and gyneacology clinic and no births can be given. The patients included in the study consisted of preterm infants born in externally and referred to our hospital. Newborns having a gestational of ≤32 weeks were included in the study. Gestational age was determined primarily through ultrasonographic evaluation in the first trimester, as well as through calculations based on the last menstrual period in pregnancies followed up on, or through clinical evaluation after delivery. Patients with congenital anomalies or malformations, metabolic disease, or who did not have family consent were barred from participating in the study. The study group consisted of newborns with a gestational age of ≤32weeks and culture proven LOS, while the control group consisted of newborns hospitalized in the NICU with a gestational age of ≤32 weeks and no signs of clinical or laboratory infection. Late-onset sepsis was defined as neonatal sepsis diagnosed between postnatal days 4 and 30 (8). Newborns with a gestational of ≤32 weeks and having a history of chorioamnionitis, premature rupture of membranes (PROM) or prolonged premature rupture of membranes (PROM ≥18 hours), maternal urinary tract infection, unknown group B streptococcus status, peripartum maternal fever, fetal tachycardia and EOS were excluded from the study. Also, preterm infants having a history of maternal anticonvulsant (phenitoin, phenobarbital), antifungal (ketoconazole), antituberculosis (rifampicin, isoniazid), anti-retroviral drug or glucocorticoid use and diseases that can affect maternal vitamin D status such as malabsorption, pancreatic insufficiency, nephrotic syndrome, cirrhosis, liver failure, hypoparathyroidism, renal failure were exluded from the study.

Blood samples for calcium (Ca), phosphorus (P), magnesium (Mg), alkaline phosphatase (ALP), parathyroid hormone (PTH), and 25-hydroxy-vitamin D (25-OHD) were obtained from all participants at postnatal six hours of life in the neonatal intensive care unit (NICU).

As no births can be given in our clinic and all the patients hospitalized in the first day of life were born in externally and referred to our hospital. So it was not possible to use cord blood for the measurement of laboratory parameters. Serum 25-OHD and PTH levels were measured by chemiluminescent immunoassay analyzer (Abbott i2000, Abbott Laboratories, USA). Ca, P, Mg and ALP levels were measured using the photometry method on the Beckman Coulter AU 680 analyzer (Danaher Corporation, Brea, CA, USA). Render BC64 device(Render Biotech Co. Ltd. Shenzen, China) was used for analyzing blood cultures.

Maternal demographic data were obtained from medical records. The maternal age at the time of delivery, presence of multiple pregnancies and concomitant maternal diseases and medications used were recorded. The characteristics of newborns, including gestational age, birth weight, sex, mode of delivery, Apgar scores, antenatal steroid use, duration of invasive and non-invasive mechanical ventilation (MV) and total parenteral nutrition (TPN), body weight at discharge, and length of hospitalization were recorded. Also, microorganisms that grew in the blood culture were recorded in the study group. Birth season was classified as summer (June, July, August), fall (September, October, November), winter (December, January, February) and spring (March, April, May). The primary focus of this study was the relationship between neonatal 25-OHD levels and development of LOS. Also, maternal 25-OHD levels and use of vitamin D supplementation could not recorded as all preterm infants included in the study were born in externally and referrred to our hospital as mentioned above.

According to neonatal 25-OHD levels, preterm infants were classified into three groups: Severe vitamin D deficiency (25-OHD levels ≤5 ng/ml), Vitamin D insufficiency (25-OHD levels 5-15 ng/ml) and normal vitamin D (25-OHD levels>15 ng/ml). 25-OHD level ≤15 ng/ml was defined as low vitamin D level (7,9).

***Statistical Analysis***

Statistical Package for Social Sciences (SPSS) 20.0 for Windows was used to evaluate the data. Qualitative variables were expressed as percentages. Continuous variables with normal distribution were expressed as mean (SD) and compared using t-test. Numerical variables that were not distributed normally were expressed as the median (Interquartile range, IQR). Kruskal Wallis tests were used for the evaluation of continuous variables, while Chi-square test was used for categorical data. Multivariable logistic regression analysis was performed to investigate the association between 25-OHD levels and LOS. A value of $p < 0.05$ was accepted as statistically significant.

## 3. Results

During the study period, 122 preterm infants having a gestational age of ≤ 32 weeks were included. From these, 58 (47.5%) had culture proven LOS (study group), while 64 (52.5%) had no signs or symptoms of sepsis (control group). There was no significant difference between the groups in terms of gestational age, birth weight, small for gestational age (SGA) infants, sex, mode of delivery, multiple pregnancy, maternal age, antenatal steroid use, accompanying maternal diseases. In contrast to that, first minute and fifth minute Apgar scores were significantly higher in the study group compared to control group. Also there was a significant difference in terms of birth season between the groups. Duration of invasive MV, non-invasive MV and TPN were statistically significant higher in the study group compared to control group. Also, study group was found to have a significantly longer length of hospital stay compared to control group (Table 1).

**Table 1.** The maternal and neonatal characteristics of the study and control groups

| Variables | Study Group n=58 | Control Group n=64 | p |
|---|---|---|---|
| **GA, week *** | 31 (30, 32) | 31 (30, 32) | 0.74 |
| **Birth weight, gr *** | 1375 (1115, 1684) | 1570 (1200, 1710) | 0.08 |
| **SGA, n (%)** | 18 (31) | 14 (21) | 0.30 |
| **Multiple pregnancy, n (%)** | 14 (24 ) | 14 (20) | 0.83 |
| **Male sex, n (%)** | 28 (48) | 32 (64) | 0.49 |
| **Use of antenatal steroid, n (%)** | 20 (31) | 28 (44) | 0.19 |
| **Delivery with CS, n (%)** | 50 (86) | 58 (90) | 0.31 |
| **Maternal age, year*** | 30 (24, 33) | 27.5 (23, 35) | 0.38 |
| **1st min Apgar*** | 8 (5, 10) | 7 (4, 9) | **0.0001** |
| **5th min Apgar *** | 9 (7, 10) | 8 (6, 10) | **0.0001** |
| **Birth season, n (%)** | | | **0.02** |
| **Summer** | 13 (22) | 3(5) | |
| **Fall** | 15 (26) | 16 (25) | |
| **Winter** | 11 (19) | 21 (32) | |
| **Spring** | 19 (33) | 24 (38) | |
| **Duration of TPN, day*** | 30 (22, 47.5) | 15 (10, 27) | **0.0001** |
| **Duration of invasive MV, day *** | 1 (0, 8) | 0 (0, 2) | **0.0001** |
| **Duration of non-invasive MV, day *** | 3.5 (0, 10.2) | 1 (0, 3.8) | **0.0001** |
| **Length of hospital stay, day *** | 49 (36.5, 67) | 31 (20, 41) | **0.0001** |
| **Body weight at discharge, gr *** | 2415 (2170, 2818) | 2250 (2050, 2490) | **0.009** |
| **Maternal disease, n (%)** | 32 (55) | 35 (55) | 0.52 |
| **Gestational Diabetes** | 3 (5) | 8 (12) | |
| **Preeclampsia** | 13 (22) | 17 (27) | |

***CS:*** *Cesarean section,* ***GA:*** *Gestational age,* ***IQR:*** *Interquartile range,* ***MV:*** *Mechanical ventilation,* ***TPN:*** *Total parenteral nutrition,* ***SGA:*** *Small for gestational age*
*** Median (Q1,Q3)***

When the groups were compared for laboratory parameters; the groups did not differ in terms of serum Ca, P, Mg, ALP, PTH levels. In contrast to that, median 25-OHD levels of study group was significantly lower than the median 25-OHD levels of the control group (10.2 ng/ml vs 18.3 ng/ml; p=0.0001). Statistically significant higher rates of low vitamin D levels (25-OHD level<15 ng/ml) were observed in the study group compared to control group (50/58, 86% vs 23/64, 36%; p=0.0001). In the study group, 7 (12%) preterm infants had severe vitamin D deficiency, 43 (%74) had vitamin D insufficiency and 8 (14%) had normal vitamin D levels. In the control group, none of the preterm infants had severe vitamin D deficiency, but 23 (36%) had vitamin D insufficiency and 41 (64%)had normal vitamin D levels (Table 2).

**Table 2.**Comparison of laboratory findings of the study and control groups

| Variables | Study Group n=58 | Control group n=64 | p |
|---|---|---|---|
| **Ca (mg/dl), mean ± SD** | 8.33 ± 0.81 | 8.52 ± 0.90 | 0.23 |
| **P (mg/dl)*** | 5.5 (4.6, 6.3) | 5.6 (5, 6.1) | 0.51 |
| **Mg (mg/dl)*** | 2.0 (1.8, 2.6) | 2.0 (1.8, 2.7) | 0.80 |
| **ALP (U/L)*** | 192 (139, 245) | 191 (155, 227) | 0.78 |
| **PTH (pg/ml)*** | 53 (32, 157) | 40 (26, 112) | 0.11 |
| **25-OHD (ng/ml)*** | 10.2 (7.2, 13.2) | 18.3 (11.5, 20.5) | **0.0001** |
| **25-OHD levels, n (%)** | | | **0.0001** |
| **Low (<15 ng/ml)** | 50 (86) | 23 (36) | |
| **Normal (≥15 ng/ml)** | 8 (14) | 41 (64) | |
| **25-OHD levels, n (%)** | | | **0.0001** |
| **Severe deficiency (≤5 ng/ml)** | 7 (12) | 0 (0) | |
| **Insufficiency( 5-15 ng/ml)** | 43 (74) | 23 (36) | |
| **Normal (≥15 ng/ml)** | 8 (14) | 41 (64) | |

***ALP:*** *Alkaline phosphatase,* ***Ca:****Calcium,* ***Mg:****Magnesium,* ***P:****Phosphorus,* ***PTH:****Parathyroidhormone,*
***25-OHD:*** *25-hydroxyvitamin D* ****Median (Q1,Q3)***

When the groups' 25-OHD levels were compared according to season, the study group's neonatal 25-OHD levels were significantly lower in all seasons compared to the control group, and this was statistically significant. Furthermore, neonatal 25-OHD levels in the control group were higher in all seasons, with the highest levels found in the summer. In contrast to that, 25-OHD levels of the study group were similar in all seasons (Table 3).

**Table 3**. Comparison of neonatal 25-hydroxyvitamin D levels in terms of season and group at birth

| Season | 25-hydroxyvitamin D level (ng/ml) | | p |
|---|---|---|---|
| | Study Group Median (min-max) | Control Group Median (min-max) | |
| **Spring** | 9.9 (4.2-18.3) | 18.8 (6.2-47.3) | **0.0001** |
| **Summer** | 9.3 (4.2-38) | 21.1 (17-26) | **0.04** |
| **Fall** | 10.4 (4.7-17.6) | 18.3 (6.5-20.5) | **0.03** |
| **Winter** | 11.7 (5.7-18.2) | 16.2 (7.1-32.3) | **0.02** |

The most common microorganism detected in blood culture was coagulase negative *Staphylococcus epidermidis* (n=28, 48%), followed by *Staphylococcus haemolyticus* (n=14, 24%), *Staphylococcus aureus* (n=6, 10%), *Klebsiella pneumonia* (n=4, 7%), *Escherichia coli* (n=4, 7%) and *Pseudomonas aeruginosa* (n=2, 4%). *Staphylococcus epidermidis* was considered as a pathogen as if it was isolated in two separate sets of blood cultures from two different sites.

Multivariable logistic regression analyses revealed that LOS development was more common in the infants with low vitamin D levels, after adjusting the effects of length of hospital stay, duration of invasive MV and TPN. When compared to preterm infants with normal 25-OHD levels, preterm infants with low 25-OHD levels were 15.2 (95% confidence interval [CI]: 5.14-45.1; p=0.0001) times more likely to experience LOS. In addition, after controlling for length of hospital stay, duration of invasive MV, and TPN, the newborn's 25-OHD level was found to be a significant predictor of LOS. Every 1-ng/mL increase in the newborn's 25-OHD level decreased the likelihood of LOS [odds ratio (OR): 0.46, 95% CI: 0.458–0.502, p=0.0001].

## 4. Discussion and Conclusion

Despite advances in neonatal care, neonatal sepsis remains a significant cause of morbidity and mortality. Neonatal sepsis and other severe infections accounted for nearly 15% of all neonatal deaths worldwide (10). With decreasing birth weight and gestational age, the risk of both EOS and LOS increases (11). Fetal distress, low Apgar scores and need for resuscitation, multiple pregnancy, EOS, frequent blood sampling, entubation, MV, invasive procedures such as catheterization and long-term TPN use especially are known to increase risk for LOS (12).

In this study, preterm infants with a gestational age of ≤32 weeks who had LOS had significantly lower 25-OHD levels than preterm infants with the same gestational age who did not have LOS. Increasing 25-OHD levels also reduces the likelihood of LOS in preterm infants with a gestational age of ≤32 weeks.

Vitamin D has a key role in calcium homeostasis, (4) but in the last years, the immune modulating effects of vitamin D on the innate and adaptive immune system have been great interest for researchers. The active form of vitamin D is 1,25-dihydroxyvitamin D and produced first by hepatic 25-hydroxylation with the cytochrome P450 2R1 and other enzymes, followed by peripheral tissue 1α-hydroxilation with CYP27B1 enzyme (4). Recent research indicates that vitamin D signaling plays an important role in immune system regulation (13). Vitamin D receptors and enzymes involved in vitamin D synthesis are abundant in immune system

cells, and pathogen detection stimulates the production of CYP27B1 via a cytokine network (14). Detection of pathogen-associated antigens and activation of pattern recognition receptors causes antimicrobial peptide production and responses including cytokines, chemokines resulting in wide signalization throughout the immune system. Antimicrobial peptide transcription is directly stimulated by vitamin D receptors bound to vitamin D. Also, vitamin D regulates anti-inflammatory response with the arrangement of dentritic cells (15).Genome-based studies on vitamin D signaling revealed that vitamin D receptors have an important role in the regulation of many different genes associated with immune system functions (16).

Compared to past few years, survival rates of preterm infants have evidently raised with advances in perinatal and neonatal care (17). As a result, rates of very low birth weight (VLBW) and extremely low birth (ELBW) infants has increased. These resulted in an increase of prematurity related morbidities and complications. Therefore, more effort has been given for the prevention rather than the treatment of these morbidities and complications. As vitamin D has many important functions in many systems in the human body, supplementation of vitamin D is given during pregnancy all over the world. In Turkey, regardless of blood 25-OHD levels, vitamin D supplementation is given beginning from the 12th week of pregnancy to end of pregnancy and continued for six months after delivery. The dose of vitamin D is 1200 IU per day given orally (18). The positive correlation of maternal and neonatal vitamin D levels is widely reported in the literature (19-22). In view of these findings, the most important strategy for preventing vitamin D insufficiency during pregnancy is to take vitamin D on a regular basis.

In light of research findings on the wide effects ofvitamin D on many systems, studies on the effect of vitamin D levels on neonatal morbidities such as respiratory distress syndrome, neonatal sepsis, and bronchopulmonary dysplasia were conducted (9,23,24). Studies evaluating the effect of vitamin D deficiency on LOS in preterm infants are very limited (5,7). A recent study evaluating neonatal and maternal vitamin D status and risk of LOS in term newborns reported that neonatal and maternal vitamin D deficiency increases risk of LOS and neonatal vitamin D is an independent predictor for LOS (21).

The study population consisted of preterm infants with a gestational age of ≤32 weeks and levels of 25-OHD were significantly lower in preterm infants with LOS and this finding was similar to results reported by Dogan et al (5). Also, consisted with previous studies evaluating the effect of vitamin D deficiency on the development of EOS in term newborns (1,24,25). In contrast to that, another study found no relationship between cord blood 25-OHD levels and neonatal sepsis in preterm infants (7).

Although, the study and control groups were similar for gestational age and birth weight, frequency of low 25-OHD levels was significantly higher in the study group. A study including preterm infants reported higher frequency of low 25-OHD levels in preterm infants with LOS similar to our results but the study group had lower gestational age and birth weight different from the present study (5). Also, multiple pregnancies are known to increase risk of LOS (12), but the study and the control groups were similar for rate of multiple pregnancy in the present study. EOS is another risk factor for LOS in newborns (12), but in the present study this data was not included.

Parenteral nutrition, existing invasive devices such as endotracheal tubes, urinary catheters, intravascular catheters and orogastric tubes increase the risk of health care associated infections especially in preterm infants. Central venous catheters and peripherally inserted catheters are commonly used for administration of parenteral nutrition. The increased risk of infections caused by the use of central lines were widely reported in the literature. Also use of lipid emulsions is an independent risk factor for bacterial or fungal sepsis (26,27). In addition to prolonged duration of MV, prolonged parenteral nutrition also increase the risk of health care-associated pneumonia in the NICU. Longer

duration of MV causes more frequent insertion of endotracheal tubes which means "to bypass" the initial host defense mechanisms such as the upper airway filtration system and mucociliary clearance system of lower respiratory tract (28). Duration of invasive MV, non-invasive MV and TPN were longer in the study group. After adjusting for the effect of these variables, low 25-OHD levels caused 15-fold increase in the risk of LOS in preterm infants. In a study, it was reported to be 7-fold increase in the risk of LOS in preterm infants (5).

Serum Ca, P, Mg, ALP and PTH levels were similar between the study and control groups in contrast to statistically significant different 25-OHD levels. One study reported lower 25-OHD levels in term newborns with EOS compared to control group but the groups did not differ for serum Ca, P, ALP levels, as our results (29).

One study evaluating the effect of low vitamin D levels on LOS in preterm infants and another study evaluating the role of vitamin D levels on the development of EOS in term infants reported no difference for birth season (5,7). In contrast to that, Ozdemir et al. reported a difference for birth season in term newborns with EOS, similar to our results (29).

Vitamin D can be synthesized from the fetal tissues but maternal vitamin D status is the most important factor on the neonatal 25-OHD levels until neonates are supported for vitamin D from external sources (30). As mentioned above, levels of 25-OHD were significantly lower in the study group compared to control group. In addition to that neonatal 25-OHD levels in the study group were significantly lower in all seasons. Because the season in which the baby is born and the traditional clothing style of women cannot be changed, the most important component is the neonatal 25-OHD level, and the lack of a seasonal effect could be linked to preterm birth, when vitamin D accumulation is highest in the third trimester (26). Cetinkaya et.al reported no difference between the study and control groups for seasonal 25-OHD levels in term newborns. Also, term newborns had significantly higher 25-OHD levels in summer compared to those born in other seasons. These findings support the relation between vitamin D synthesis and exposure to sunlight (1). Our finding was similar with that results. To our knowledge, this is the first study comparing the seasonal 25-OHD levels of preterm infants with LOS and those without LOS.

The present study focused on the relation between neonatal 25-OHD levels and the development of LOS. This study has several limitations. Firstly, due to its retrospective nature, the maternal 25-OHD levels at the time of delivery were not evaluated. Secondly, pregnant women are given vitamin D supplementation beginning from the 12th week of gestation. In the present study, the use of vitamin D supplementation (no usage, irregular use, regular use) was not included. As exposure to sunlight is the most important factor for vitamin D synthesis and use of sun-protective clothing is a major factor this process, these were not included in the study. Another limitation was the small sample size of the study population.

Preterm infants with LOS were found to have significantly lower 25-OHD levels compared to preterm infants at the same gestational age without LOS and low 25-OHD levels seem to increase risk of neonatal LOS. Up to now, there is no established optimal 25-OHD level for adequate immune function for preventing neonatal sepsis in both term and preterm infants. Further studies with larger sample size are needed to achieve precise results. Besides, taking into account the potential negative maternal and neonatal effects of vitamin D deficiency appropriate supplementation should be administered in regions where vitamin D deficiency is frequent.

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