The relation between vitamin D level and increased risk for early-onset neonatal sepsis in full-term infants
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Objectives The aim of the study was to determine the role of maternal and neonatal plasma vitamin D levels in early-onset sepsis (EOS) and in its severity in term infants.

Methods This study was performed on 60 neonates and their mothers who were divided into two groups. Group 1 comprised 30 full-term neonates with EOS. Group 2 comprised 30 healthy neonates of matched age and sex who served as the control group. All participants were subjected to the following investigations: complete blood count, C-reactive protein, blood culture and serum vitamin D levels in both neonates and their mothers.

Results Maternal and neonatal vitamin D levels were lower in the study group compared with the control group and their levels were significantly higher in the summer season. We found a significant negative correlation between infant vitamin D level and C-reactive protein in the patient group. In contrast, there was a significant positive correlation between maternal and neonatal vitamin D levels in both groups. The best cutoff value of neonatal vitamin D for the risk of sepsis was 14.4 ng.

Conclusion Lower maternal and neonatal vitamin D levels are associated with EOS. However, there was no significant relation between vitamin D deficiency and outcome. Med Res J 15:16–21 © 2016 Medical Research Journal.

Methods The study was conducted at Neonatal Intensive Care Units of the Pediatric Department at Benha University Hospital and Benha Teaching Hospital during the period from December 2014 until August 2015. Informed consent was taken from the parents before enrollment of their neonates in the study. The study was approved by the Ethical Committee of the Pediatric Department of Benha University Hospitals, Benha, Egypt. This study was conducted on 60 neonates and their mothers, who were divided into two groups.

Group 1 included 30 full-term neonates with EOS and their mothers.
Group 2 included 30 full-term healthy neonates and their mothers with no clinical or laboratory evidence of sepsis.

The patient group was screened for the following inclusion and exclusion criteria.

Inclusion criteria
Neonates had to be full-term infants (>37 week), of either sex, and with clinical and laboratory findings of EOS according to the criteria defined by Gitto et al. [12].
Exclusion criteria

Being preterm, presence of risk factors such as chorioamnionitis, premature rupture of the membrane, intrapartum fever, or urinary tract infection in the mother, refusal of parental consent, lack of laboratory data, and major congenital anomalies in the infant constituted the exclusion criteria.

All participants were subjected to the following:

1. Full history taking.
2. Full clinical examination.
3. Laboratory investigations, including:
   1. A complete blood picture using an automated hematology system (Sysmex XE 5000; Sysmex Corporation, Japan) [13].
   2. C-reactive protein (CRP) using latex agglutination (CRP-Latex Cromatest, Mexico).
   4. Serum 25(OH)D levels in both infant and mother using DRG 25(OH)D (total) ELISA EIA-5396 kits according to the manufacturer’s instructions.

Season of birth was classified into three groups during the study period: winter (December, January and February), spring (March, April and May), and summer (June, July and August). Maternal vitamin D supplementation was classified in terms of usage: no usage, insufficient usage (total usage < 3 months) and regular usage (total usage >3 months).

The patient group was followed up during their hospital stay period and for 30 days after discharge from the Neonatal Intensive Care Unit for determining the sepsis outcome.

Statistical analysis

The collected data were tabulated and analyzed using SPSS, version 16 software (SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as number and percentages, whereas quantitative data were expressed as mean ± SD and range. The χ²-test and Fisher’s exact test were used to analyze categorical variables. Continuous variables were tested for normality using the Kolmogorov–Smirnov test. The Student ‘t’ test was used to compare the mean of two parametric variables and the Mann–Whitney U-test was used for nonparametric data. The Kruskal–Wallis test was used to compare three nonparametric variables. Spearman’s correlation coefficient (r) was used to test the correlations between two nonparametric variables. The receiver operating characteristic (ROC) curve was used to detect the cutoff value of vitamin D level with optimum sensitivity and specificity in the prediction of early-onset neonatal sepsis. The accepted level of significance in this work was stated at 0.05 (P < 0.05 was considered significant).

Results

The patient group comprised 30 newborns: 21 boys (70%) and 9 girls (30%), with a mean gestational age of 38.0 ± 0.7 weeks and mean birth weight of 2.9 ± 0.4 kg. The control group comprised 30 healthy, full-term newborns: 15 boys (50%) and 15 girls (50%), with a mean gestational age of 38.4 ± 0.97 weeks and a mean birth weight of 3.03 ± 0.14 kg. No significant difference was found between the two groups in terms of sex, birth weight and gestational age.

In the patient group, eight (26.7%) neonates were delivered vaginally, and 22 (73.3%) neonates were delivered by cesarean section (CS). In the control group, 18 (60%) neonates were delivered vaginally, and 12 (40%) neonates were delivered by CS. There was a significant difference between the two groups in terms of birth season and mode of delivery.

In our study there was a highly significant difference between the patient group and the control group regarding maternal educational status, being significantly lower in the patient group. In addition, the number of mothers in the patient group who never and/or irregularly used vitamin supplementation was significantly higher than that in the control group (P < 0.001) (Fig. 1).

In our study there was a highly statistically significant difference as regards both neonatal vitamin D level in the EOS group during different seasons, being significantly higher in the summer season than in winter and spring (P < 0.001) (Table 1).

Also, there was a highly statistically significant difference between patients and controls as regards serum vitamin D level, being significantly higher in the control group and their mothers than in the patient group and their mothers (Fig. 2).

In our study, we found a significant negative correlation between infant vitamin D level and CRP in the patient group (r = -0.455, P = 0.011) (Fig. 3). A significant positive correlation was detected between maternal and neonatal vitamin D levels in both groups. (r = 0.883, P < 0.001, in the EOS group; r = 0.485, P = 0.007, in the control group.)

ROC analysis of the data showed that the best cutoff value for neonatal vitamin D for the risk of sepsis was 14.4 ng (sensitivity = 96.7%; specificity = 96.7%; area under the curve = 0.93; negative predictive value = 96.7; and positive predictive value = 96.7), whereas the cutoff maternal vitamin D was 28.25 ng (sensitivity = 96.7%; specificity = 96.7%; area under the curve = 0.96; negative predictive value = 96.7; and positive predictive value = 96.5) (Table 2 and Fig. 4).

Discussion

Neonatal septicemia remains one of the main causes of mortality and morbidity despite the progress in hygiene, introduction of new and potent antimicrobial agents for treatment, and advanced measures for diagnosis. It is responsible for 30–50% of total neonatal deaths in developing countries [2]. In the current study it was found that the vaginal mode of delivery was significantly associated with increased frequency of sepsis. This may be related to good sterilization and intrapartum chemo-
prophylaxis, which dramatically decreased the risk for sepsis in neonates delivered by CS.

In the current study, there was a significant difference between the study group and the control group regarding season of birth. We found that about 50% of our cases was born in winter, as there were no other risk factors for occurrence of sepsis in these selected cases. This high incidence may be attributable to vitamin D deficiency in this season. This is in agreement with the findings of Cetinkaya et al. [14], who observed that the incidence of infection and sepsis was highest during winter months as circulating vitamin D is mainly derived from synthesis in the skin under the influence of sunlight, depending on season and latitude.

In our study, there was a highly significant difference between the patient and control groups regarding maternal education status. We found that about 53% of the mothers in the septic group were not educated and about 43% were educated to less than high school. This agrees with the reports of Cetinkaya et al. [14], who observed that lower educational status was associated with increased risk for infection, which could be attributed to the lower vitamin D level in this group because of less awareness about vitamin D supplementation during pregnancy. Pehlivan et al. [15] also found a positive correlation between serum 25(OH)D level and educational status. However, an earlier study in Pakistan by Atiq et al. [16] found lower serum 25(OH)D levels in mothers and their infants belonging to the high socioeconomic group and related that to preference of women of high socioeconomic group to live indoors with reduced exposure to direct sunlight.

In our study there was a highly significant difference between the study group and the control group regarding the history of vitamin D intake by mothers. We found that the number of mothers in the patient group who never and/or irregularly used vitamin supplementation was significantly higher than that in the control group. This is in agreement with the results of Cetinkaya et al. [14], who observed that the lower vitamin D level was associated with insufficient intake of vitamin D as vitamin D status of the newborn at birth is primarily dependent on the vitamin D status of the mother during pregnancy. Pehlivan et al. [15] found that infant vitamin D status is more affected directly by sunshine exposure of the mother than by her nutritional intake. The vitamin D stores of the newborn depend entirely on the vitamin D stores of the mother. If the mother is vitamin D-deficient, the infant will be deficient because of decreased maternofetal transfer of vitamin D. Also the blood 25(OH)D concentrations in the neonates correlated well with the maternal 25(OH)D concentrations [17].
In our study, we found a highly significant difference between maternal vitamin D level and infant vitamin D level, being significantly lower in patients with sepsis compared with healthy controls. Previous studies also found a significant association between vitamin D deficiency and sepsis suggesting that vitamin D status is an important contributor to sepsis incidence and outcome [18–20].

In the present study, we found a significant negative correlation between infant vitamin D level and CRP. This is in agreement with the studies by Tao et al. [21] and Chen et al. [22], who showed that vitamin D supplementation significantly decreased the circulating CRP levels. Another trial showed that vitamin D supplementation during pregnancy resulted in a significant decrease in maternal serum CRP [23]. Liefaard et al. [24] also confirmed that serum vitamin D was inversely associated with CRP, but Grzanka et al. [25] did not observe any significant association between concentrations of 25(OH)D and CRP. This may be due to the small sample size and single assessment of 25(OH)D concentrations performed in the summer.

In our study, we found a highly significant difference in infant and maternal vitamin D levels in different seasons, with higher levels in summer. This agrees with the observations of Amrein et al. [26] who found that vitamin D status was surprisingly dependent on the season with a maximum difference of a factor of two between the lowest levels in or after winter and the highest levels in August.

Our study showed a nonsignificant difference between sepsis outcome and vitamin D status in the patient group. This is in agreement with the observations by Morominzato et al. [27], who could not find a causative link between hypovitaminosis D and outcomes. Other studies by Braun et al. [28] and Amrein et al. [26], however, revealed that low serum concentrations of 25(OH)D were associated with adverse clinical outcomes, including increased mortality rate, increased length of hospital stay, and acute kidney injury in a critical care setting not limited to severe infection and sepsis. Also Rech et al. [29] revealed that deficiency in 25(OH)D and 1,25(OH)2D was a significant predictor of 30-day mortality in septic patients.

ROC analysis of our data showed that the best cutoff value for neonatal serum 1,25(OH) is 14.4 ng and that for maternal serum 1,25(OH)D is 28.25 ng for prediction of neonatal sepsis. Infant vitamin D had a sensitivity of 96.7%, specificity of 96.7% and area under the curve of 96%, whereas maternal vitamin D had a sensitivity of 93.3%, specificity of 96.7% and area under the curve of 93%. From these data, we suggest that vitamin D is of good predictive value for prediction of early-onset neonatal sepsis.

**Conclusion**

25(OH)D levels were found to be associated with seasons, regular intake of vitamin D and socioeconomic status. Low maternal 25(OH)D levels were found to be associated with low educational level. Lower maternal and neonatal 25(OH)D levels are associated with EOS. Adequate vitamin D supplementation during pregnancy may be helpful in prevention of EOS in term neonates.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

الملخص العربي

العلاقة بين نسبة فيتامين D وزيادة معدلات حدوث التسمم الدموي المبكر في الأطفال كاملي النمو

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كان هدف هذا العمل وجود علاقة بين نسبة فيتامين D عند المواليد والأمهات وحدوث التسمم الدموي المبكر في المواليد كاملي النمو. وقد أجريت هذه الدراسة على 60 طفل حديثي الولادة وأمهاتهم تنتمون إلى مجموعتين: مجموعة المرضى وهما 30 طفلاً، مجموعة المراقبة وهم 30 طفل كاملي النمو، يعانون من تسمم دموي مبكر وأمهاتهم. المجموعة المراقبة وهم 30 طفل كاملي النمو لا يعانون من تسمم دموي مبكر وأمهاتهم. تم أخذ التاريخ المرضي الكامل والفحص الشامل لجميع المراقبة وأجريت التحاليل الألية لهم: تعداد الدم الكامل (CRP)، مزعة الدم، نسبة فيتامين D عند كل من الأم والطفل وقد أشتملت المجموعة المرضية على 30 مولود: 21 نفر (70%) و9 نفر (30%)، ومتوسط عمر الرحمي (8.0 ± 71، متوسط الوزن عند الولادة (2.9 ± 0.40 كجم، واستلمت المجموعة المراقبة على 30 مولود: 15 من الذكور (50%) و15 من الإناث (50)، وكان متوسط العمر الرحمي من 38.4 ± 0.97 أسبوع. ومتوسط الوزن عند الولادة (3.6 ± 0.14 كجم). وواجت تعداد المرضي بنمو 8 أطفال بنسبة (26.7%) ولدتهم طبيعاً عن طريق المهبل، و22 من الأطفال بنسبة (73.3%) ولدتهم بعملية قيصرية. وفي مجموعتي المراقبة يوجد 18 نساء (60%) من الأطفال حديثي الولادة ولدتهم طبيعاً عن طريق المهبل، 12 نساء (40%) من الأطفال ولدتهن بطريقة قيصرية، وكانت نتائج الدراسة بان كلاً: لا توجد اختلافات ذات قيمة إحصائية بين المجموعتين فيما يتعلق بجنس الطفل والوزن عند الولادة والطول والرقم. وتوجد اختلافات ذات إحصائية بين المجموعتين بالنسبة لطريقة الولادة طبيعية أم قيصرية وفي أي قسم كانت الولادة (شأنها أو رباعي أو صغير). والنسبة للتاريخ المرضي للأم، توجد اختلافات ذات دلالة إحصائية كبيرة جداً بين المجموعتين بالنسبة لدرجة تطعيم الأم، وما إن كانت إلا اختلاف فيتامين D أثناء الحمل لا. ويوجد اختلافات ذات دلالة إحصائية بين المجموعتين بالنسبة لفيتامين D عند كل من الأطفال وأمهاتهم. وقد وجدت علاقة سلبية بين نسبة فيتامين D والبروتينات التفاعلي م في الأطفال المرضي، وواجد علاقة إيجابية وثابل بين مستويات فيتامين D عند الأم والطفل في كل المجموعتين. ووجد أنه لا توجد علاقة بين نسبة فيتامين D ونتائج المراقبة. ووجد أيضاً أنه لا علاقة له مع صبر الطفل من حيث النفع أو الوفاة، ووجد علاقة ذات دلالة إحصائية عالية جداً بين نسبة فيتامين D في الأطفال وميقولة النسبة، وأظهر البالانون أن القمة الفاصلة فيتامين D في الظروف تحت حدوت التسمم الدموي المبكر هو 14.4 وانجح لمحاسبي ROC للبيانات. نسب التسمم الدموي (6.7%) للفحص الفاصلة فيتامين D عند الأم واختبار حدوت التسمم الدموي هو 8.28% (انجح لمحاسبي) لفحص التسمم الدموي (93.3%) (انجح لمحاسبي 96.7%). وتم استنتاج من النتائج أن نسبة فيتامين D عند المواليد وأمهاتهم يقترن بزيادة حدوث تسمم دموي مبكر في هؤلاء الأطفال.