Association between vitamin D level and community-acquired late-onset neonatal sepsis

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ABSTRACT

Introduction. The objective was to determine the relationship between mother and infant vitamin D levels and late onset sepsis.

Population and methods. Infants born ≥37 weeks of gestational age who were hospitalized with the diagnosis of late-onset sepsis were enrolled to this prospective case control study. Vitamin D levels of the infants and their mothers in the study and a control group were compared.

Results. Fourty six term patients with late-onset sepsis composed the study group, 46 patients with hyperbilirubinemia as the control group. Vitamin D supplementation during pregnancy was lower in mothers of study group compared to the control group (p = 0.001). Serum 25-hydroxyvitamin D levels of infants and mothers in the study group were significantly lower than the control group (p < 0.001). There was a positive correlation between 25-hydroxyvitamin D levels of mothers and infants in both groups (r: 0.38, p < 0.001). The best cut off value of 25-hydroxyvitamin D, which determines the risk of late-onset sepsis in neonates, was detected as 15.45 ng/ml (sensitivity: 91.3 %, specificity: 71.7 %, area under the curve: 0.824, p < 0.001).

Conclusions. In this study, 25-hydroxyvitamin D levels were found to be lower in term infants with late-onset sepsis and among their mothers compared to the control group. Positive correlation was found between serum 25(OH)D levels of infants and their mothers.

Key words: newborn infant, sepsis, vitamin D, pregnancy.

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INTRODUCTİON

Neonatal sepsis is a clinical syndrome with systemic signs and symptoms of infection in the first month of life and a specific agent isolated in the blood culture. Despite advances in neonatology, it is still an important cause of mortality and morbidity. The frequency of sepsis is between 1-8.1 per 1000 live births.[1] Neonatal sepsis can be classified into three groups according to the onset of symptoms as early, late and very late onset sepsis (LOS).[2] Late onset sepsis is defined as sepsis diagnosed at 4-30th days of life. In developed countries, coagulase-negative staphylococci are the most common causative agents in LOS, whereas gram-negative bacilli (*E.coli, Klebsiella, Pseudomonas*) are more common in developing countries.[3] Late onset sepsis may develop with vertical passage as neonatal colonization and subsequent infection, or with horizontal passsage from caregivers and environmental factors.

In addition to the well-known classical effects of vitamin D on calcium and bone metabolism, it has been suggested to play a role in the pathogenesis of many diseases such as cancer, multiple sclerosis, diabetes and cardiovascular diseases through cell proliferation and immune functions.[4] Vitamin D regulates both natural and adaptive immune systems. Vitamin D affects the innate immune system by inducing antimicrobial peptides in epithelial cells, neutrophils and macrophages.[5] The hormonal metabolite of vitamin D, 1,25-dihydroxyvitamin D, induces the production of human cathelicidin LL-37 with antimicrobial and antiendotoxin effects which contribute to innate immune response

to sepsis.[6] Also, the relationship between vitamin D deficiency and lower respiratory tract infections has been demonstrated in children and neonates.[7,8] It has been shown that low vitamin D levels in adults and children may result with increased sensitivity to sepsis.[9-11]

The most important reason of vitamin D deficiency in newborns is the lower maternal vitamin D levels.[12] Maternal serum 25 (OH) D levels in pregnancy is associated with 25 (OH) D levels of the cord blood and the newborn.[13-15] As breast milk is a weak vitamin D source, maternal vitamin D levels has an important effect on health and growth in neonatal and early childhood period.[13]

The objective of this study was to determine the relationship between mother and infant vitamin D levels and LOS.

POPULATION AND METHODS

Infants born $\geq$ 37 weeks of gestational age who were hospitalized with the diagnosis of LOS in our newborn intensive care unit between January 1, 2017 and December 31, 2018 were enrolled to this case-control study. The control group was selected from patients born $\geq$ 37 weeks of gestational age who were hospitalized for hyperbilirubinemia after postnatal 72 hours of age. Patients with hospital acquired infections, pneumonia, congenital abnormalities, congenital heart defects, genetic diseases, babies in the first 72 hours of life, those previously hospitalized for infection and refugees were excluded from the study.

The study protocol was approved by the local Ethics Committee (26.12.2016/2016-101). Informed consent was obtained for all infants from their parents.

According to European Medicines Agency (EMA) criteria, presence of at least two out of 6 clinical categories and at least two out of 6 laboratory categories were considered as clinical sepsis.[16]

EMA sepsis score

Clinical findings: 1. Body temperature > 38.5 °C or < 36 °C. 2. Respiratory: Apnea, tachypnea, increased oxygen and ventilation need. 3. Cardiovascular: Bradycardia, tachycardia or rhythm disorder, urinary output < 1 ml/kg/h, hypotension, impaired peripheral perfusion. 4. Gastrointestinal: Nutritional intolerance, poor suction, abdominal distention. 5. Skin or subcutaneous lesions: Petechia, scleremia. 6. Non-

specific: Irritability, lethargy, hypotonicity.

Laboratory findings: 1. White blood cell (WBC) count < 4000/mm^3 or > 20,000/mm^3. 2. Immature/total (I/T) neutrophil: $\geq$ 0.2. 3. Platelet count: < 100,000/mm^3. 4. C-reactive protein (CRP) > 1.5 mg/dl or procalcitonin $\geq$ 2 ng/ml. 5. Glucose intolerance at least 2 times: Hyperglycemia (> 180 mg/dl) or hypoglycemia (< 45 mg/dl). 6. Metabolic acidosis: Base excess <−10 mMol/l or serum lactate > 2 mMol/l.

Birth date, birth weight, mode of delivery, gestational week and gender of the babies were recorded. Maternal age, number of pregnancies, diseases during pregnancy (chorioamnionitis, gestational diabetes, premature rupture of membranes, urinary tract infection, preeclampsia, eclampsia), vitamin D supplementation in pregnancy, mother's educational level, consanguineous marriage and scarf use were examined. Serum 25(OH)D levels of the mothers were also measured simultaneously with the infants. In Turkey, 1200 IU vitamin D supplementation per day has been recommended to all pregnants from 12 weeks of gestation to 6 months after birth since 2011. Vitamin D supplementation in pregnancy was divided into 3 groups: 1. None 2. Irregular intake (totally < 6 months). 3. Regular intake. Mothers who had low serum 25(OH)D levels were referred to general practitioners for treatment. Infants' previous hospitalizations, age at diagnosis, indwelling catheters, stay on mechanical ventilator, antibiotic treatment, inotropic use and duration of treatment were recorded.

Hemoglobin, hematocrit, WBC, I/T neutrophil ratio, CRP, calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) values of all infants with suspected LOS were evaluated. Blood, urine, cerebrospinal fluid (CSF) cultures of all babies in the study group were obtained before initiating antimicrobial therapy. Hemogram, I/T neutrophil ratio, CRP, blood culture control, and also CSF and urine cultures if the first examined ones were positive, were repeated after 48-72 hours of treatment.

Diagnosis of urinary tract infection (UTI) was made if $\geq$ 10,000 CFU/ml single microorganism growth in catheter urine culture. Urinary system was evaluated by renal USG among all patients with diagnosis of UTI. The diagnosis of meningitis was made by biochemical and microbiological evaluation of CSF obtained by lumbar puncture.

Serum 25(OH)D levels of infants diagnosed with sepsis and their mothers were evaluated

within 72 hours. Serum 25(OH)D levels were measured by electrochemiluminescence (ECLIA) method using Beckman Coulter hormone autoanalyser.

IBM SPSS Statistics 17.0 program was used for statistical analysis. Shapiro-Wilk test was used to determine whether the variables were compatible with normal distribution. Median (Interquartile Range, IQR) was used for descriptive statistical analysis of variables that were not normally distributed, and mean ± SD (Standard Deviation) for the analysis of variables with normal distribution. Chi-square test was used for categorical data and Mann Whitney U test was used as non-parametric test for numerical data that did not show normal distribution. Pearson correlation test was used for correlation between maternal and infant vitamin D levels. The receiver operating characteristic (ROC) curve was used to determine the cut-off value of 25(OH)D level with optimum sensitivity and specificity in predicting LOS. In this study, the level of significance was accepted as $p < 0.05$.

RESULTS

A total of 1137 patients were hospitalized in our neonatal intensive care unit during the study period. Of these patients, 175 infants were diagnosed as sepsis according to EMA criteria. A total of 82 patients were excluded from the study because of having early onset sepsis. Of the remaining patients, 47 of 93 patients with LOS were excluded from the study due to nasocomial sepsis, premature birth, pneumonia, renal abnormality and being refugees. While 46 term patients with community acquired LOS composed the study group (*Figure 1*), 46 patients were selected from patients with hyperbilirubinemia as the control group.

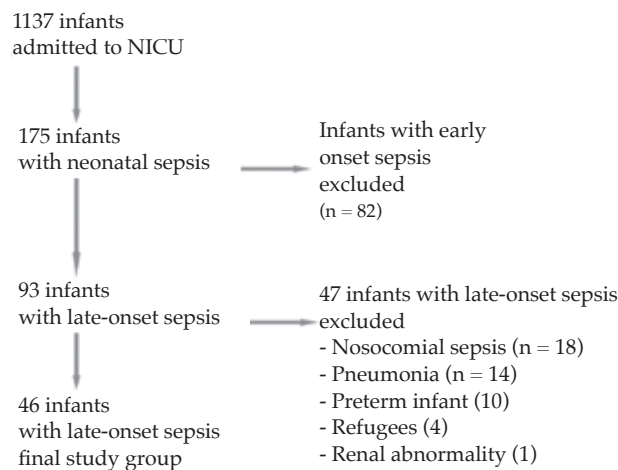FIGURE 1. *Flow diagram of the study group*

TABLE 1. *Demographic characteristics of study and control groups*

	Study group (n = 46)	Control group (n = 46)	*p*
Birth weight (g), mean ± SD	3213 ± 500	3135 ± 368	0.395
Gestational age, mean ± SD	38.7 ± 1.4	38.2 ± 1.4	0.413
Gender			
Female, n (%)	20 (43.5)	18 (39.1)	0.672
Male, n (%)	26 (56.5)	28 (60.9)	
Delivery type			
Normal, n (%)	17 (37)	23 (50)	0.207
C/S, n (%)	29 (63)	23 (50)	
Previous hospitalization, n (%)	9 (19.6)	3 (6.5)	0.063
Age at admission, (days) mean ± SD	14.3 ± 4.8	10.5 ± 5.4	0.001
Sibling presence, n (%)	33 (71.7)	24 (52.1)	0.053

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

The mean birth weight of 46 infants in the study group was 3213 ± 500 grams and the mean gestational age was 38.7 ± 1.4 weeks. In the study group, 56.5 % of the babies were male and 63 % of them were born by cesarean section. Birth weight, gestational week, gender, mode of delivery, previous hospitalization and sibling presence were similar between the groups (p > 0.05). There was a significant difference between the groups for age at admission (p = 0.001). All demographic data is summarized in *Table 1*. Maternal age, pregnancy–related diseases (gestational diabetes, hypertension, preeclampsia), mother's

educational levels, scarf use and consanguineous marriage rates were similar between the groups (p > 0.05). Vitamin D supplementation during pregnancy was lower in mothers of the study group compared to the control group (p = 0.001) (*Table 2*).

The clinical presentation of LOS is shown in *Table 3*. The microorganisms isolated in the blood cultures of the patients were as follows: 10 gram positive microorganisms (5 group B *Streptococcus*, 3 *Streptococcus pneumoniae*, 2 *Enterococcus spp*), and 4 gram negative microorganisms (2 *Klebsiella pneumoniae*, 2 *Escherichia coli*). Urine cultures

FIGURE 2. *The receiver operating characteristic (ROC) curve to predict the cut-off value of 25-hydroxyvitamin D level for late onset neonatal sepsis*
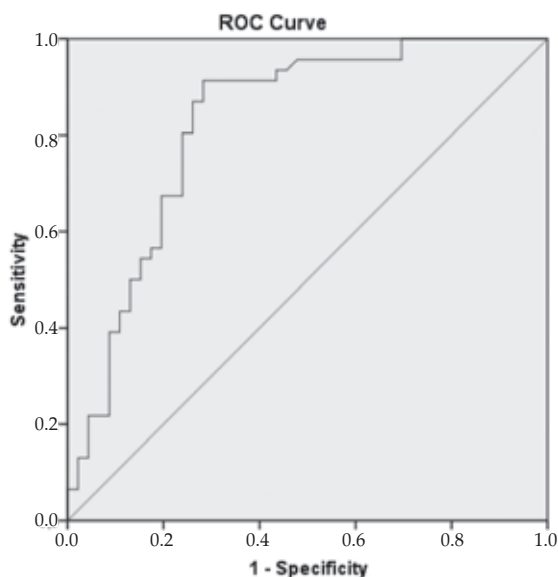

TABLE 2. *Demographic characteristics of the mothers*

	Study group (n = 46)	Control group (n = 46)	p
Maternal age, mean ± SD	27.30 ± 6.25	28.33 ± 6.5	0.456
Perinatal comorbidity, n (%) (GDM, HT, etc.)	5 (10.8)	1 (2.1)	0.315
Education level, n (%)			
No education	2 (4.3)	3 (6.5)	
Primary school graduation	25 (54.3)	14 (30.4)	0.074
High school graduation	16 (34.8)	22 (47.8)	
University graduation	3 (6.5)	7 (15.2)	
Scarf use, n (%)	33 (71.7)	32 (69.6)	0.819
Consanguinity, n (%)	4 (8.7)	7 (15.2)	0.335
Vitamin D supplementation during pregnancy, n (%)			
None	26 (56.5)	7 (15.2)	
Irregular usage	18 (39.1)	32 (69.6)	0.001
Regular usage	2 (4.3)	7 (15.2)	

GDM, gestational diabetes; HT, hypertension; SD, standard deviation.

detected gram negative microorganisms (12 *Escherichia coli*, 2 *Klebsiella pneumoniae*, 1 *Pseudomonas aeruginosa)* in 15 patients and gram positive microorganism (1 *Enterococcus spp*, 1 *Streptococcus pneumoniae*) in two patients. *Staphylococcus aureus* was detected in CSF culture of one patient. *Coagulase-negative* staphylococci was isolated in the blood culture of three patients but since the control blood culture of these patients were negative, it was accepted as skin contamination.

In the study group, there were no patients on mechanical ventilation, none received inotropic therapy and no mortality was detected. When the laboratory findings of the groups were compared, hemoglobin, hematocrit and WBC counts were different between the groups (p < 0.05), while platelet count, Ca, P and ALP levels were similar (p > 0.05) (*Table 4*).

Serum 25(OH)D levels of infants and their mothers in the study group were significantly lower than the control group (p < 0.001) (*Table 4*). There was a positive correlation between 25(OH)D levels of mothers and infants in both groups (r: 0.38, p < 0.001). The best cut off value of 25(OH)D in ROC analysis was determined as 15.45 ng/ml

with 91.3 % sensitivity and 71.7 % specificity (AUC = 0.824, 95 % confidence interval 0.737-0.912, p < 0.001) for predicting LOS (*Figure 2*).

DISCUSSION

In this prospective observational study, serum 25(OH)D levels were found to be lower in term infants with community-acquired LOS and among their mothers compared to the control group. Positive correlation was found between serum 25(OH)D levels of infants and their mothers. The best cut off value of vitamin D, which determines the risk of LOS was detected as 15.45 ng/ml (sensitivity: 91.3 %, specificity: 71.7 %, area under the curve: 0.824, p < 0.001). Vitamin D levels below 15.45 ng/ml may increase the risk of LOS in newborns. Therefore, providing vitamin D supplementation to pregnant women with low vitamin D levels and to their babies after birth may have an effect on preventing infections in the neonatal period.

Vitamin D is known for years as a fat-soluble steroid hormone that plays a role in calcium homeostasis and maintaining skeletal mineralization. In the last decade, vitamin D receptor (VDR) was detected and also vitamin D-activating enzyme 1-alpha hydroxylase (CYP27B) was shown in tissues other than the kidneys, such as immune system cells, intestines, pancreas and prostate. Thus, in addition to the known classical effects of vitamin D, it has been suggested that especially active vitamin D has similar properties to local active cytokines.[17] Because the adaptive immunity in newborns is not well developed, protection against pathogens

TABLE 3. *The clinical presentation of late-onset sepsis*

	Study group, n (%)
Primary bloodstream infection	14 (30.4)
Urinary tract infection	17 (37)
Meningitis	1 (2.2)
Clinical sepsis	14 (30.4)

TABLE 4. *Laboratory results of the infants*

	Study group (n = 46)	Control group (n = 46)	p
Hemoglobin (g/dl) mean ± SD	14.8 ± 2.3	16.2 ± 2.1	0.020
Hematocrit (%), mean ± SD	44 ± 6.9	48.9 ± 6.6	0.010
WBC count (/mm³), mean ± SD	16,658 ± 5163	10,726 ± 2080	0.000
Trombocyte count (/mm³), mean ± SD	316,152 ± 127,382	311,450 ± 91,011	0.839
Ca (mg/dl), mean ± SD	9.7 ± 0.7	9.9 ± 0.7	0.060
P (mg/dl), mean ± SD	6.1 ± 1.1	6.1 ± 1.1	0.891
ALP (U/l), mean ± SD	237 ± 100	259 ± 90	0.277
Immature/total neutrophil, mean ± SD	0.31 ± 0.1	na	
CRP (mg/dl), mean ± SD	5.98 ± 1.03	na	
Neonatal 25 (OH) D (ng/ml), mean ± SD	12.9 ± 6.36	21 ± 6.38	<0.001
Maternal 25 (OH) D (ng/ml), mean ± SD	9.8 ± 6.07	15.3 ± 4.71	<0.001

ALP, alkaline phosphatase; Ca, calcium; CRP, C-reactive protein; na: not applicable; P, phosphorus; SD, standard deviation; WBC, white blood cell; 25 (OH) D, 25-hydroxyvitaminD.

is mainly influenced through the innate immune system.[18] Vitamin D has a complex effect on immune functions and enhances innate immunity. Since vitamin D is used as a substrate for the production of antimicrobial peptides such as cathelicidin, it can play an important role in preventing infections during pregnancy and early childhood.[19] In a recent study, vitamin D deficiency was reported to be associated with decreased lymphocyte subgroups and changes in T-lymphocyte activation, which may increase the risk of infection in newborns.[20] In our study, the supplementation of vitamin D in pregnancy was found to be lower in the mothers of infants diagnosed with sepsis compared to the control group. In studies from Turkey, vitamin D levels of pregnant women were found as 4.97-14.82 ng/ml.[21-23] Although our country is mostly sunny; cultural reasons, covered dressing, shorter daily sunbathing periods may cause low vitamin D levels in women.

The Society of Endocrinology defines serum 25(OH)D levels $\leq$ 20 ng/ml as vitamin D deficiency.[24] According to this classification, vitamin D levels were found to be deficient in the mothers of both groups (9.8 ng/ml vs. 15.3 ng/ml). Vitamin D deficiency is highly prevalent worldwide; and its prevalence differs between 9 % - 24 % to 36 % - 90 % in healthy children living in high-income countries versus middle/low-income countries.[25] A recent meta-analysis has shown that 25(OH)D deficiency in acute and critically ill children is high and associated with increased mortality.[25] Also, positive correlation was found between 25(OH)D levels of mothers and their babies. Similar studies showed correlation between maternal and cord blood 25(OH)D levels.[13-15] Vitamin D levels of newborns seems to be dependent on mothers' reserves; but The World Health Organization does not recommend routine vitamin D supplementation during pregnancy.[26] There is insufficient evidence for routine vitamin D supplementation in pregnancy to improve maternal and infant outcomes in the 2016 Cochrane meta-analysis.[27] Although vitamin D supplementation of 1200 IU per day to all mothers from 12 weeks of gestation up to 6 months after birth has been recommended in Turkey since 2011, the rate of regular vitamin D supplementation during pregnancy was low in both groups in our study. In regions where vitamin D deficiency is common, such as our country, vitamin D supplementation may be recommended to pregnant women.

In our study, while the most common clinical presentation of LOS was urinary tract infections the following presentations were primary bloodstream infections and clinical sepsis. In a Swiss study, the prevalence of primary bloodstream infection was 45 % and urinary tract infection was 32 % in community acquired LOS.[28] Blood culture is the gold standard for the diagnosis of neonatal sepsis, but culture negative sepsis is responsible for most episodes in developing countries.[29] Lower determination of primary bloodstream infections in our study may be due to technical deficiencies for isolating agents in blood culture. Therefore, sepsis diagnosis was based on clinical and laboratory findings.

In our study, serum 25(OH)D levels of infants with LOS were found to be lower than the control group (study group: 12.9 $\pm$ 6.36 ng/ml vs. control group: 21 $\pm$ 6.38 ng/ml; p <0.001). The cut off value of 25(OH)D, which determines the risk of LOS in newborns, was shown to be 15.45 ng/ml. The relationship between vitamin D levels and early-onset neonatal sepsis was first demonstrated by two studies conducted in 2015.[10,30] In the first study, Çetinkaya et al., stated that serum 25(OH)D levels were lower in infants and their mothers in the early sepsis group compared to the control group.[10] In the second one, Cizmeci et al., showed an increased risk of early-onset newborn sepsis in infants with low cord-blood 25(OH)D levels.[30] After these studies showing the effect of vitamin D in early-onset sepsis, similar studies were performed for LOS. In an Indian study, 25(OH)D levels were measured in infants with LOS; and similar to our study, 25(OH)D levels in infants and their mothers were found to be lower in the study group compared to the control group (study group: 15.4 ng/ml vs. control: 21.4 ng/ml; p = 0.001).[11] Unlike our study, late premature babies and also patients hospitalized with pneumonia were also included. In another study among 175 term infants with culture positive LOS, the mean 25(OH)D level was found to be lower than the healthy control group (sepsis group:12.28 ng/ml vs. control: 14.88 ng/ml; p = 0.002).[31] Low 25(OH)D levels both in the mother and in the infant may be a risk factor for neonatal infections. Community-based LOS still remains an important problem in developing countries. Low neonatal vitamin D levels, which is correlated with maternal 25(OH)D concentrations, may increase LOS risk in newborns. With regular vitamin D supplementation and sunbathing recommendations during pregnancy, adequate vitamin D levels can be achieved in infants. Thus,

it may be possible to contribute to the prevention of LOS in the newborn period. Further studies are needed to demonstrate the effects of vitamin D on neonatal infections.

Limitations of our study are as follows: 1. Serum 25(OH)D levels of the infants in the study group were obtained during the infection but could not be measured before and after the infection period. 2. The control group was selected from patients with the diagnosis of hyperbilirubinemia, so the age at admission was lower than the study group. 3. The rate of culture proven sepsis was low due to technical inadequacy. 4. The sample size was small.

In conclusion, in this study, 25-hydroxyvitamin D levels were found to be lower in term infants with late-onset sepsis and among their mothers compared to the control group. Positive correlation was found between serum 25(OH)D levels of infants and their mothers. ∎

REFERENCES

1. Nizet V, Klein J. Bacterial Sepsis and Meningitis. In: Wilson C, Nizet V, Maldonado Y, Remington J, Klein J (eds). *Remington and Klein's Infectious Disease of the Fetus and Newborn Infant*. 8 ed. Philadelphia: Elsevier Saunders; 2016.Pags.217-71.

2. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? *Lancet*. 2005; 365(9462):891-900.

3. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. *Arch Dis Child Fetal Neonatal Ed*. 2015; 100(3):F257-63.

4. Valdivielso JM, Fernández E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta*. 2006; 371(1-2):1-12.

5. Clancy N, Onwuneme C, Carroll A, McCarthy R, et al. Vitamin D and neonatal immune function. *J Matern Fetal Neonatal Med*. 2013; 26(7):639-46.

6. Mookherjee N, Rehaume LM, Hancock RE. Cathelicidins and functional analogues as antisepsis molecules. *Expert Opin Ther Targets*. 2007; 11(8):993-1004.

7. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoğlu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. *Eur J Clin Nutr*. 2009; 63(4):473-7.

8. Muhe L, Lulseged S, Mason KE, Simoes EA. Case control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. *Lancet*. 1997; 349(9068):1801-4.

9. Upala S, Sanguankeo A, Permpalung N. Significant association between vitamin D deficiency and sepsis: a systematic review and meta-analysis. BMC Anesthesiol. 2015; 15:84.

10. Cetinkaya M, Cekmez F, Buyukkale G, Erener-Ercan T, et al. Lower vitamin D levels are associated with increased risk of early-onset neonatal sepsis in term infants. *J Perinatol*. 2015; 35(1):39-45.

11. Dhandai R, Jajoo M, Singh A, Mandal A, Jain R. Association of vitamin D deficiency with an increased risk of late-onset neonatal sepsis. *Paediatr Int Child Health*. 2018; 38(3):193-7.

12. Ergür AT, Berberoğlu M, Atasay B, Şıklar Z, et al. Vitamin D deficiency in Turkish mothers and their neonates and in women of reproductive age. *J Clin Res Pediatr Endocrinol*. 2009; 1(6):266-9.

13. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. 2011; 26(10):2341-57.

14. Khalesi N, Bahaeddini SM, Shariat M. Prevalence of maternal vitamin D deficiency in neonates with delayed hypocalcaemia. *Acta Med Iran*. 2012; 50(11):740-5.

15. Thorne-Lyman A, Fawzi W. Vitamin D during pregnancy and maternal, neonatal and infant health outcomes: asystematic review and meta-analysis. *Pediatr Perinat Epidemiol*. 2012; 26 (Suppl 1):75-90.

16. Europen Medicines Agency. Report on the Expert Meeting on Neonatal and Pediatric Sepsis. London; EMA, 2010. [Accessed on: January 13, 2020]. Available at: https://www.ema.europa.eu/en/documents/report/report-expert-meeting-neonatal-paediatric-sepsis_en.pdf.

17. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients*. 2013; 5(7):2502-21.

18. Firth MA, Shewen PE, Hodgins DC. Passive and active components of neonatal innate immune defenses. *Anim Health Res Rev*. 2005; 6(2):143-58.

19. Chesney RW. Vitamin D and The Magic Mountain: the anti-infectious role of the vitamin. *J Pediatr*. 2010; 156(5):698-703.

20. Youssef MAM, Zahran AM, Hussien AM, Elsayh KI, et al. In neonates with vitamin D deficiency, low lymphocyte activation markers are risk factors for infection. *Paediatr Int Child Health*. 2018; 39(2):111-8.

21. Halicioglu O, Aksit S, Koc F, Akman SA, et al. Vitamin D deficiency in pregnant women and their neonates in spring time in western Turkey. *Paediatr Perinat Epidemiol*. 2012; 26(1):53-60.

22. Parlak M, Kalay S, Kalay Z, Kirecci A, et al. Severe vitamin D deficiency among pregnant women and their newborns in Turkey. *J Matern Fetal Neonatal Med*. 2015; 28(5):548-51.

23. Özdemir AA, Ercan Gündemir Y, Küçük M, Yıldıran-Sarıcı D, et al. Vitamin D Deficiency in Pregnant Women and Their Infants. *J Clin Res Pediatr Endocrinol*. 2018; 10(1):44-50.

24. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, et al. Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011; 96(7):1911-30.

25. Cariolou M, Cupp MA, Evangelou E, Tzoulaki I, Berlanga-Taylor AJ. Importance of vitamin D in acute and critically ill children with subgroup analyses of sepsis and respiratory tract infections: a systematic review and meta-analysis. *BMJ Open*. 2019; 9(5):e027666.

26. WHO recommendations on antenatal care for a positive pregnancy experience. Geneva: World Health Organization, 2016. [Accessed on: January 13, 2020]. Available at: https://apps.who.int/iris/bitstream/handle/10665/250796/9789241549912-eng.pdf;jsessionid=FEC7D02BC085AEA4D2C49209397A0E66?sequence=1.

27. De-Regil LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev*. 2016; (1):CD008873.

28. Giannoni E, Agyeman PKA, Stocker M, Posfay-Barbe KM, et al. Swiss Pediatric Sepsis Study. Neonatal Sepsis of Early Onset, and Hospital-Acquired and Community-Acquired Late Onset: A Prospective Population-Based Cohort Study. *J Pediatr*. 2018; 201:106-14.e4.

29. Thaver D, Zaidi AKM. Burden of neonatal infections in developing countries: a review of evidence from community-based studies. *Pediatr Infect Dis J*. 2009; 28(1

Suppl):S3-9.

30. Cizmeci MN, Kanburoglu MK, Akelma AZ, Ayyildiz A, et al. Cord-blood 25-hydroxyvitamin D levels and risk of early-onset neonatal sepsis: a case control study from a tertiary care center in Turkey. *Eur J Pediatr*. 2015; 174(6):809-15.

31. Agrawal A, Gupta A, Shrivastava J. Role of Vitamin-D Deficiency in Term Neonates with Late-Onset Sepsis: A Case-Control Study. *J Trop Pediatr.* 2019; 65(6):609-16.