Research Article

Relation between Vitamin D Level and Some Inflammatory Cytokines in Full-Term Newborns with Early Onset Sepsis

Marwa Ahmed Abd El-kader*[1], Gamal Abd El-khalek El-Azab [2], Shymaa Mohamed El-Rifaey [3]

1. Teaching assistant of clinical pharmacy, Department of Clinical Pharmacy, Faculty of Pharmacy, Sinai University, North Sinai, El-Arish, Egypt.
2. Assistant professor of clinical pharmacy, Department of Clinical Pharmacy, Faculty of Pharmacy, Tanta University, Tanta, Egypt.
3. Lecturer of pediatrics, Department of pediatrics, Faculty of Medicine, Tanta University, Tanta, Egypt.

***Corresponding author's E-mail:** marwa_ahmed464@yahoo.com

Received: 05-03-2018; **Revised:** 30-03-2018; **Accepted:** 14-04-2018.

ABSTRACT

Neonatal sepsis remains a serious problem with a high mortality and morbidity rate in neonates especially in developing countries. The present study was designed to investigate the possible association between vitamin D level and inflammatory cytokines such as Interleukin-6 (IL-6) and C-reactive protein (CRP) in neonates with early onset sepsis (EOS). This study was conducted on fifty full-term neonates who, divided into 25 neonates diagnosed with EOS based on the clinical and laboratory findings (study group) and 25 healthy neonates with no signs of infection (control group). Blood samples were obtained within the first 72 hours of the neonate's life for measuring of CRP and IL-6 for all neonates in the two groups.25-hydroxyvitamin D (25-OHD) level was measured for all neonates and their mothers in the two groups. Our results indicated that, the mean maternal and neonatal 25-OHD (22.30/8.56 ng /ml respectively) were significantly lower than the control group (36.04/28.55 ng/ml respectively, p-value < 0.001), the mean IL-6 level (198.07 pg/ml) was significantly higher in the study group than the control group (24.71 pg/ml, p-value < 0.001). The cutoff value of neonatal 25-OHD was ≤ 11.5 ng/ml and for maternal 25-OHD was ≤ 29.12 ng/ml. A positive correlation between maternal and neonatal 25-OHD levels was detected, a significant negative correlation between 25-OHD level and IL-6 level in neonates with EOS. Our study showed a significant inverse relation between 25-OHD level and IL-6 level so adequate vitamin D level could decrease the risk of EOS in neonates.

Keywords: 25-hydroxyvitamin D; C-reactive protein; Early Onset Sepsis; Interleukin-6.

INTRODUCTION

Neonatal sepsis is the presence of signs and symptoms of infection with suspected or proven bacteremia in the first 30 days of life, it is a common disease with high morbidity and mortality rate.[1,2] Neonatal sepsis may be divided into three types: early, late and very late- onset neonatal sepsis. [3] Early-onset sepsis is mainly associated with transferring of the infectious agents from mother to neonate within the first 72h of life. [1,4] Foul smelling, low birth weight, meconium-stained liquor, prematurity, premature rupture of membranes, prolonged labor, and perinatal asphyxia are considered the main risk factors for EOS.[1,5]

Vitamin D is a fat-soluble steroid hormone which has a part in the upkeep of ordinary calcium level and skeletal development.[6] Vitamin D also has an immunomodulatory impact on neonates [7], It may contribute in the advancing of the functions of the immune system by induction the generation of antimicrobial peptides from epithelial cells, macrophages, and neutrophils. [7, 8] Many in-vitro studies have reported that vitamin D supplementation inhibits the pro-inflammatory cytokine generation in many cells like macrophages and monocytes. [9]

CRP is a nonspecific marker for inflammation, neonatal CRP >10 mg/L was related to many adverse clinical conditions such as sepsis. [10] IL-6 is a cytokine produced by monocytes, endothelial cells, and fibroblasts.[11] IL–6 level in cord blood was elevated in chorioamnionitis, fetal infection, and funisitis. [12] The detection limit of IL-6 is >100 pg/ml. [13] IL-6 is the main inducer of hepatic synthesis of proteins as CRP. [14-16] As IL-6 regulates the transition from innate to acquired immunity through induction of acute phase protein production in the liver such as CRP and neutrophil mobilization from bone marrow and blood vessels.[17, 18]

This study was designed to evaluate the possible effect of vitamin D level on inflammatory cytokines such as IL-6 and CRP in Egyptian neonates with EOS. We present convincing evidence regarding the possible effects of vitamin D as a protective agent in full term neonates against EOS.

SUBJECTS AND METHODS

Study design and patients' populations

This is a single-center, prospective study was conducted on 50 neonates and their mothers. They were divided into two groups. Study group consisted of 25 full-term neonates who were diagnosed to have a high probable EOS according to the criteria defined by **Gitto et al**. [19] Control group consisted of 25 healthy neonates with the same age and sex as the study group. For all studied groups a complete history was taken including, gestational age, birth weight, sex, mode of delivery, Apgar scores and the birth season. A questionnaire was designated to obtain information from mothers including age, educational level, the presence of disease and the use of maternal sun protective clothes. Seasons of birth

were classified into three classifications: December, January and February which is the winter, March, April, and May which is the spring, June, July and August which is the summer. Vitamin D deficiency was staged as severe deficiency (serum25-OHD < 10 ng/ml), insufficiency (serum 25-OHD between 11 and 32ng/ml), and adequate (serum 25-OHD between 32 and 100 ng /ml).[20] All neonates recruited between March 2016 and November 2017. from the Pediatrics Department, Tanta University Hospital. Neonates of the study group should be a full - term neonates (>37 weeks of gestational age), with a high probable sepsis according to the criteria defined by **Gitto et al**.[19] and of either sexes. Neonates were excluded from the study if they have risk factors such as having chorioamnionitis, premature rupture of the membrane, intrapartum fever, lack of laboratory data, neonates with probable or possible sepsis according to the criteria defined by **Gitto et al.** [19] Major congenital anomalies and being preterm.

Sample collection and preparation

Blood samples were collected from all neonates and their mothers within the first 72 hours of the neonates' life, before initiating antimicrobial therapy. Blood was gathered, permitted to cluster and then serum was obtained by centrifugation at 3000 rpm for 10 min. All samples were stored frozen at $_{-80}$°C till the analysis.

Biochemical markers

IL-6 and 25-OHD were tested using appropriate ELISA kits. Serum level of IL-6 was obtained from PicoKine™ (Pleasanton, USA) for all neonates of both groups. Serum 25-OHD levels for all neonates and their mothers of both groups were obtained from DRG Diagnostics (25-OHD total) ELISAEIA-5396kits (Marburg, Germany). Both tests were done according to the protocol provided by the manufacturers. Whole blood count (using an automated hematology system), CRP (using latex agglutination), and cultures were studied immediately these parameters were determined using commercial kits from Spectrum Diagnostics (Cairo, Egypt).

Ethical Approval

The investigation protocol of this study was in accordance with the ethical standards of ethical committee at College of Pharmacy, Tanta University; and Tanta University Hospital Institutional Review Board research committee. The study was conducted in conformity with the standards of Good Clinical Practices and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained for all neonates from their parents before including them in the study.

Statistical analysis

Data were analyzed using SPSS software, version 16.0 (Chicago, IL, USA) and presented as mean ± standard deviation (SD). The chi-square test was used to compare categorical variable between study and control groups. The differences between groups in this study were analyzed using the unpaired t-test for continuous variables. Analysis of variance (one-way ANOVA) was used to indicate the relation between season and 25-OHD level. The cut-off values of IL-6 and Vitamin D with ideal specificity and sensitivity in the diagnosis of EOS was calculated using Receiver Operating Characteristic (ROC) curve. A significant difference was assumed for $P < 0.05$.

RESULTS

Demographic and clinical characteristics

This study included 50 full-term neonates. From those neonates,25 had EOS (study group) in which the mean gestational age and birth weight were 38.5 ±0 .5 weeks and 2.7± 0.4 kg, respectively.14 neonates (56%) were male and 60% of the study group was born via cesarean section. No significant difference was found between the two groups in terms of sex, gestational age, mode of delivery, maternal age and Apgar scores. In the study group birth weight was lower than the controls, most of the neonates were born on Winter. perinatal co-morbidities and the use of maternal sun protective clothes were significantly higher in the study group than the control group. The educational status of mothers was significantly low in the study group, mothers who never used vitamin D and those who used it irregularly during pregnancy were significantly higher in the study group than the control group. In the study group 88 % neonates had a sever 25-OHD deficiency (<11 ng/ml) which was significantly lower than the control group.

(Table 1) showed that the white Blood Cells (WBCs), IL-6 and CRP levels were significantly higher in the study group than the control group (p-value < 0.001 in all), while platelet count was significantly lower in the study group than the control group (p-value <0.001). Both maternal and neonatal 25-OHD levels were significantly lower in the study group than the control group.

(Table 2) showed that maternal and neonatal 25-OHD levels of the study group were lower in all seasons than the control group, a markedly significant increase was found in maternal and neonatal 25-OHD levels in summer in the study group than in other seasons.

Table (3) showed that the cutoff value of neonatal 25-OHD was ≤11.5 ng/ml (sensitivity =100%; specificity =96; negative predictive value (NPV)=100; and positive predictive value (PPV)= 96.2), while the cutoff value of maternal 25-OHD was ≤ 29.12 ng /ml (sensitivity =100%; specificity =100%; NPV =100; and PPV =100).The best cutoff point of IL-6, CRP for diagnosis of neonatal sepsis were >109.85 pg/ml and > 2.99 mg/dl respectively while the sensitivity of IL-6, CRP were 92%, 100% respectively, specificity of IL-6 was 100 %, specificity of CRP was 64% .

Table 1: Biochemical tests result of the two studied groups

	Groups		T-Test	
	Study group, n=25	Control group, n=25		
	Mean ± SD	Mean ± SD	t	P-value
WBC (10^3/ml)	16195 ± 5.518	9320 ±1324	6.058	< 0.001*
Platelet count (10^3/ml)	121798±24630	304789 ± 24550	21.564	< 0.001*
C-RP (mg /dl)	14.78±9.3	5.1±3.6	4.767	< 0.001*
IL-6 (pg/ml)	198.074 ±59.58	24.71±35.46	12.500	< 0.001*
Neonatal vitamin D (ng/ml)	8.562±2.180	28.550±3.046	-26.683	<0.001*
Maternal vitamin D (ng/ml)	22.300±5.047	36.047±1.243	-13.223	<0.001*

C-RP =C-reactive protein, **IL-6**=Interleukin-6 &**W.B. Cs**= White Blood Cells, **SD**=standard deviation

* Representing significant difference, P- value < 0.05.

Table 2: Neonatal and maternal vitamin D levels in the study group in different season of birth

	Season of birth			ANOVA
	Winter, n =12	Spring, n= 7	Summer, n= 6	
	Mean ± SD	Mean ± SD	Mean ± SD	P-value
Neonatal vitamin D (ng/ml)	7.423 ± 2.055	8.353 ± 1.440	11.087 ± 0.442	0.474[a],**0.001***[b],**0.018***[c]
Maternal vitamin D (ng/ml)	9.575 ± 3.928	22.727 ± 5.081	27.253 ± 3.136	0.263[a],**0.003***[b],0.142[c]

[a] Comparison of Winter with Spring; [b] Comparison of Winter with Summer; [c] Comparison of Spring with Summer; SD=standard deviation; * Representing significant difference (P- value < 0.05).

Table 3: Validity and predictivety of vitamin D, IL-6 and CRP levels in the prediction of early-onset neonatal sepsis

Variable	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
Neonatal vitamin D (ng/ml)	≤11.5	100.00	96	96.2	100.00	99%
Maternal vitamin D (ng/ml)	≤29.12	100.00	100.00	100.00	100.00	100%
IL-6 pg/ml	>109.85	92.00	100.00	100.00	92.6	99%
CRP mg/dl	>2.99	100.00	64	73.5	100.00	86.2%

CRP=C-reactive protein, **IL-6** = interleukin-6, **NPV**= negative predictive value, **PPV**= positive predictive value, **Sens** =sensitivity; **Spec**=specificity.

Table 4: IL-6, CRP and neonatal vitamin D with regard to blood culture outcome in the study group

	Blood culture outcome						T-Test	
	No growth			Growth				
	Mean	±	SD	Mean	±	SD	t	P-value
IL-6 pg/ml	157.967	±	37.315	258.234	±	24.466	-7.467	<0.001*
CRP mg/dl	14.876	±	10.069	14.639	±	8.756	0.061	0.952
Neonatal vitamin D ng/ml	10.057	±	1.258	6.320	±	0.946	7.989	<0.001*

CRP=C-reactive protein, **IL-6** = interleukin-6; **SD**=standard deviation; * significant difference, P- value < 0.05.

(Table 4) showed that IL-6 was significantly higher in neonates with culture-proven sepsis than the neonates without culture-proven sepsis, while there is no significant difference between negative blood culture and positive blood culture in the study group with regard to CRP level. Neonatal 25-OHD levels were a significantly lower in neonates with culture-proven sepsis than neonates without culture-proven sepsis.

Correlations between neonatal 25-OHD with maternal 25-OHD, IL-6

(Figure 1) showed a positive correlation was observed between maternal and neonatal 25-OHD levels . (Figure 2) showed a significant inverse relation between vitamin D and IL-6. On contrast there was a non-significant relation between vitamin D and CRP.

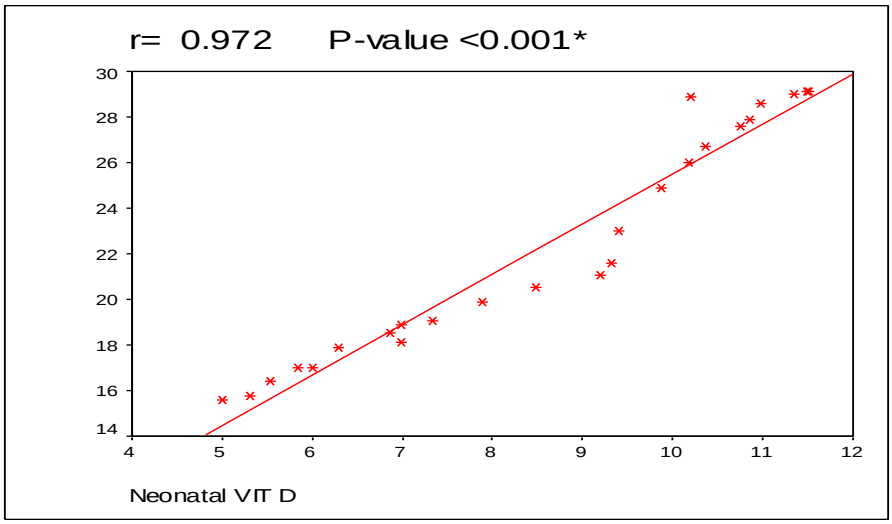

Figure 1: Positive correlation between maternal and neonatal vitamin D level

VIT D (vitamin D ng/ml); * Representing significant difference, P- value < 0.05.

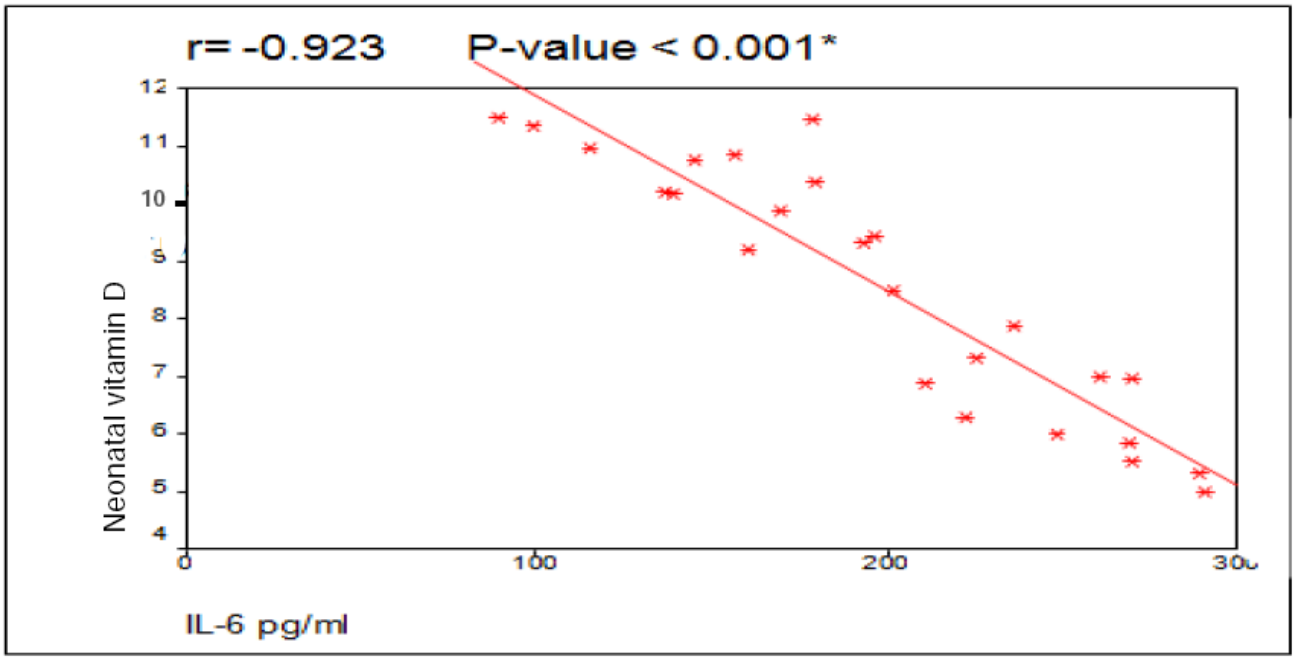

Figure 2: Negative correlation between IL-6 level and neonatal vitamin D level.

IL-6 =interleukin-6 ; VIT D (vitamin D ng/ml); * Representing significant difference, P- value < 0.05.

DISCUSSION

Septicemia in neonates remains one of the major causes of morbidity and mortality although the advancement inherent in hygiene and development of potent new antimicrobial agents. 30–50% of death in neonates in developing countries happens due to neonatal sepsis. [21]

In this study 48% of neonates in the study group was born on winter, so the high rate incidence may happen due to vitamin D deficiency in winter as there is no other cause for sepsis, this finding is similar to what Cetinkaya et al [22], Amrein et al, [23] and Seliem et al. [24] observed, because the circulating vitamin D which is mainly come from the exposure of skin to sunlight was suspected to be low in

this season. The number of mothers who preferred to wear sun protective clothes was significantly higher in the study group than the control group this like the results found by Cetinkaya et al. [22] Maternal education status of the study group was significantly lower than mothers of the control group, this is similar to the results found by Cetinkaya et al. [22] and Seliem et al. [24]

On the other hand, Atiq et al. [25] noticed that maternal and neonatal serum 25-OHD levels were low in a population with high socioeconomic status, and thought that it may be due to that pregnant woman of high socioeconomic class prefer to live indoors with limited exposure to direct sunlight. perinatal co-morbidities like preeclampsia, gestational diabetes were significantly higher in the study group than the mothers of the control group, this opposes with what Cetinkaya et al. [22] found.

Mothers of the study group who never used vitamin D and those who used it irregularly during pregnancy were significantly higher than the mothers of the control group, this similar to the findings of Cetinkaya et al. [22] and Seliem et al. [24] who noted that lower vitamin D level was related to insufficient supplementation of vitamin D. On contrast Pehlivan et al. [26] found that neonatal vitamin D status is more affected by the mother's sun exposure than by her vitamin supplementation.

This study found that neonatal and maternal vitamin D levels were lower in the study group than the control group, this goes with many studies which found a relation between vitamin D deficiency and infection. [22,24,27,28] A Positive correlation was detected between maternal and neonatal 25-OHD levels, this similar with the results of Cetinkaya et al. [22] and Seliem et al.[24] In the study group, neonates who were born on summer had a significantly higher level of 25-OHD compared with those who were born in other seasons. This was similar to the findings of Cetinkaya et al. [22] and Seliem et al. [24]

In the study group 88 % neonates had a sever 25-OHD deficiency (<11 ng/ml) which was significantly lower than the control group this result is close to those of Cetinkaya et al. [22] who found that 84% of the neonates with EOS had a sever 25-OHD deficiency.

The cutoff value for neonatal 25-OHD was 11.5 ng/ml and for maternal 25-OHD was 29.1 ng/ml. Neonatal vitamin D had a sensitivity of 100 %, specificity of 96 % whereas maternal vitamin D had a sensitivity of 96%, specificity of 100 % which close to the results of Gamal et al. [29] who had found that the cut-off value was < 8.01 ng/ml for neonatal 25-OHD was<16.82 ng/ml for maternal 25-OHD. The sensitivity, specificity, PPV and NPV were 84%, 79%, 94.7% and 82.3% respectively for neonatal and 82%, 77%, 91.4% and 80.6% for maternal 25-OHD, respectively , also Seliem et al. [24] showed that the cutoff value for neonatal vitamin D was 14.4 ng/ml and that for maternal vitamin D was 28.25ng/ml which may predict neonatal sepsis with 96.7% sensitivity, 96.7% specificity for neonatal vitamin D while maternal vitamin D had 93.3% sensitivity, 96.7% specificity from these data we can recommend that the determination of vitamin D level may be a good tool for predication of early-onset neonatal sepsis.

This study assessed the relation between vitamin D deficiency and the result of blood culture in EOS and noted that neonatal and maternal 25-OHD levels were significantly lower in culture-proven sepsis than newborns without culture-proven sepsis, this differs from the results of Cetinkaya et al. [22] who revealed that there was no significant difference in the result of blood culture with regard to vitamin D level.

IL-6 has been one of the most widely studied pro-inflammatory cytokines due to its role as an infectious marker in neonatal sepsis.[30,31] This study showed that IL-6 level was significantly higher in study group than the control group, this was similar to earlier study done by Panero et al.[32]

The cut-off value of IL-6 obtained in our study was >109.85 pg/ml ,92 % sensitivity,100 %specificity ,100% PPV,92.6% NPV and 99% accuracy. This may confirm the findings of Sonawane et al. [33] who showed that the cutoff value of IL-6 was >100 pg/ml,95.83% sensitivity and 87.50% specificity while PPV and NPV were 92% and 93.33% respectively, Kumar et al .[11] found that IL-6 had a sensitivity of 87% and specificity of 80.1%, PPV and NPV were 80.5% and 81%, respectively.

IL-6 level was significantly higher among neonates with positive blood culture than neonates with negative blood culture while CRP level was non-significantly different in terms of blood culture results. These results corresponded with Kumar et al. [11] who showed that the sensitivity of IL-6 was higher than CRP among neonates with positive blood culture.

In the present study, we found a significant negative relation between neonatal vitamin D level and IL-6 level, Zasloff et al. [34] found an evidence that vitamin D has a regulatory role on immune system. Wobke et al.[35] Also, showed there is an association between vitamin D deficiency and a wide range of diseases. Several studies have examined the relationship between vitamin D supplementation and serum levels of inflammatory markers such as CRP and cytokines. Barker et al. [36] demonstrated a positive association with interferon-γ, [IFN]-γ and IL-10. Peterson et al. [37] demonstrated a significant inverse correlation between vitamin D and serum tumor necrosis factor [TNF]-α. However other studies noted that no association found with CRP, IL-6, or IL-10 nor marked changes in serum levels of IL-4, IL-5, IL-10, IL-13, IL-2, IL-6, [IFN]-γ, and TNF-α .[38,39]

Study limitations/Recommendations

This study was self-funded and the small sample size was the main study limitation, but we considered this study as a pilot one providing evidence that vitamin D level is negatively correlated with IL-6. We suggest performing

future meta-analysis study in order to reach unanimous &clinically relevant conclusion.

CONCLUSION

From the results of the present study we can conclude that vitamin D level is negatively correlated with IL-6. No significant correlation between vitamin D and CRP. IL-6 may be an early promising biomarker with a high sensitivity and a good specificity for sepsis, especially if combined with CRP to confirm each other's. Low neonatal 25-OHD level was associated with sepsis especially with culture-proven sepsis so it can be used as a prognostic biomarker for blood culture outcome. 25-OHD levels in neonates were associated with maternal levels, season of birth, and regular intake of vitamin D, educational level and socioeconomic status of the mother.

Acknowledgements: We are grateful to the participants who had a great role in this study and to the staff members of neonatal intensive care unit, Tanta university Hospital for their support in collecting data.

REFERENCES

1. Sankar JM, Agarwal R, Deorari AK, Paul VK, Sepsis in the newborn, Ind Journal of Pediatrics, 75, 2008, 261–266 ,DOI:101007212098-008-0056.
2. Ng PC, Lam HS, Diagnostic markers for neonatal sepsis, Curr Opin Pediat, 18, 2006, 125–131, DOI: 10109701000019329387022.
3. Lawn JE, Cousens S, Zupan J, Neonatal survival 4 million neonatal deaths: When? Where? Why?, Lancet, 365, 2005, 891–900, DOI:10101601406736(05)710485.
4. Stoll BJ, Hansen NI, Sánchez PJ, Faix RG, Poindexter BB, Van Meurs KP, Matthew J, Bizzarro, Ronald N, Goldberg, Ivan D, Frantz, Ellen C, Hale, Seetha Shankaran, Kathleen Kennedy, Waldemar A, Carlo, Kristi L, Watterberg, Edward F, Bell, Michele C. Walsh, Kurt Schibler, Abbot R, Laptook, Andi L, Shane, Stephanie J, Schrag, Das A,Higgins RD, Early onset neonatal sepsis: the burden of group B streptococcal and E. coli disease continues, Pediatrics, 127, 2011, 817 –826, DOI:10.1542.2010-2217.
5. Schuchat A, Zywicki SS, Dinsmoor MJ, Mercer B, Romaguera J, O'Sullivan MJ, Patel D, Peters MT, Stoll B, Levine OS, Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study, Pediatrics, 105, 2000, 21 –26,DOI:10.1542105121.
6. De Luca HF, Overview of general physiologic features and functions of vitamin D, American Journal of Clinical Nutrition, 80, 2004, S1689–S1696, DOI: 10109380616895.
7. Clancy N, Onwuneme C, Carroll A, McCarthy R, McKenna MJ, Murphy N ,Molloy EJ, Vitamin D and neonatal immune function, J Matern Fetal Neonatal Med, 26, 2013, 639–646, DOI:10310914767058201274630 4.
8. Kempker JA, Han JE, Tangpricha V, Ziegler TR, Martin GS, Vitamin D and sepsis: an emerging relationship, Dermato endocrinol, 4, 2012, 101 –108,DOI:10.4161/derm.19859.
9. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, Goleva E,Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1, J Immunol, 188, 2012, 2127–2135, DOI:10.4049/jimmunol.1102412.
10. Hofer N, Zacharias E,Müller W, Resch B, An update on the use of C-reactive protein in early-onset neonatal sepsis: Current insights and new tasks, Neonatology, 102, 2012, 25–36, DOI:10.1159/000336629.
11. Kumar N, SinghMK, DayalR, GuptaS, GargR, Diagnostic Value of IL-6 in Neonatal Sepsis, Annals of Applied Bio-Sciences, 3, 2016, A67-71, DOI:123123/32132181.
12. Arnon S, Litmanovitz I, Regev R, Lis M, Shainkin-Kestenbaum R, Dolfin T, The prognostic virtue of inflammatory markers during late onset sepsis in preterm infants, J Perinat Med, 32, 2004, 176-80, DOI:10.1515/JPM.2004.032.
13. Milenia Biotec ,Interpretation guide for immune monitoring: 2013 update .Available from: http://annamed.co.uk/Sepsis_Neonatal.pdf
14. Martin RJ, Fanaroff AA, Walsh MC, Postnatal bacterial infections, Neonatal–Perinatal Medicine: diseases of the fetus and infant, 8th, 2, Elsevier Mosby, Philadelphia, 2006, 791-805.
15. Shelonka RL, Freij B J, Mc Crachen GH, Bacterial and fungal infections. In: Avery GB, Fletcher MA, MacDonald MG. Neonatology: pathophysiology & management of the newborn, 6th, Lippincott Williams & Wilkins, Philadelphia, 2005, 1235-47.
16. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, Pacifico L, C-reactive protein, interleukin–6 and Procalcitonin in the immediate postnatal period: influence of illness severity risk status antenatal and perinatal complications and infection, Clin Chem, 49, 2003, 60-8, DOI: 10.1373/49.1.60.
17. Orlikowsky TW, Neunhoeffer F, Goelz R, Eichner M, Henkel C, Zwirner M, Poets CF,Evaluation of IL- 8concentrations in plasma and lysed EDTA-blood in healthy neonates and those with suspected early onset bacterial infection, Pediatr Res, 56, 2004, 804–809, DOI: 10.120301.0000141523.68664.
18. Laborada G, Rego M, Jain A, Guliano M, Stavola J, Ballabh P, Krauss AN, Auld PA, Nesin M, Diagnostic value of cytokines and C-reactive protein in the first 24 hours of neonatal sepsis, Am J Perinatol, 20, 2003, 491–501,DOI: 10.1055/s-2003-45382.
19. Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, Cordaro S, Corona G, Trimarchi G, Barberi I, Effects of melatonin treatment in septic newborns, Pediatr Res, 50, 2001, 756–760, DOI:10.1203/00006450-200112000-00021.
20. Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C, Implications of vitamin D deficiency in pregnancy and lactation, Am J Obstet Gynecol, 202, 2010, 429e1–429ee, DOI:10.1016/j.ajog.2009.09.002.
21. Naher HS, Khamael AB, Neonatal sepsis: the bacterial causes and the risk factors, Int Res J Medical Sci, 1, 2013, 19–22, DOI:3.ISCA-IRJMedS-2013-027,
22. Cetinkaya M, Cekmez F, Buyukkale G, Erener-Ercan T, Demir F, Tunc T, Aydın FN, Aydemir G, Lower vitamin D levels

are associated with increased risk of early-onset neonatal sepsis in terminfants, J Perinatol, 35, 2015, 39–45, DOI:10.1038/jp.2014.146.

23. Amrein K, Zajic P, Schnedl C, Waltensdorfer A, Fruhwald S, Holl A, Purkart T, Wünsch G, Valentin T, Grisold A, Stojakovic T, Amrein S, Pieber TR, Dobnig H,Vitamin D status and its association with season, hospital and sepsis mortality in critical illness, Crit Care, 18, 2014, R47, DOI:10.1186/cc13790.
24. Seliem MS, Abdel Haie OM, Mansour AI, Salama SS,The relation between vitamin D level and increased risk for early-onset neonatal sepsis in full-term infants, Med Res J, 15, 2016, 16–21,

    DOI:10.1097/01.MJX.0000483971.52646.4b.
25. Atiq M, Suria A, Nizami SQ, Ahmed I, Maternal Vitamin-D deficiency in Pakistan, Acta Obstet Gynecol Scand, 77, 1998, 970–973, DIO:199849839.
26. Pehlivan I, Hatun S, Aydogan M, Babaog ˘lu K, Go ¨kalp AS, Maternal vitamin D deficiency and vitamin D supplementation in healthy infants,Turk J Pediatr, 45, 2003, 315–320, DOI:154914768796.
27. Upala S, Sanguankeo A, Permpalung N,Significant association between vitamin D deficiency and sepsis, BMC Anesthesiol, 15, 2015, 84, DOI:10.1186/s12871-015-0063-3.
28. Youssef DA, Ranasinghe T, Grant WB, Peiris AN, Vitamin D's potential to reduce the risk of hospital-acquired infections, Dermato endocrinol, 4, 2012, 167–175, DOI:10.4161/derm.20789.
29. Gamal TS, Madiha AS,Hanan MK, Abdel-Azeem ME,Marian GS,Neonatal and Maternal 25-OH Vitamin D Serum Levels in Neonates with Early-Onset Sepsis, children, 4, 2017, 37, DOI:10.3390/children4050037.
30. Ng PC, Lam HS, Biomarkers for late-onset neonatal sepsis: Cytokines and beyond, Clin Perinatol, 37, 2010, 599–610, DOI:10.1016/j.clp.2010.05.005.
31. Arnon S, Litmanovitz I, Diagnostic tests in neonatal sepsis, Curr Opin Infect Dis, 21, 2008, 223–7, DOI: 10.109700133282.
32. Panero A, Pacifico L, Rossi N, Mancuso G, Stegagno M, Chiesa C, Interleukin-6 in neonates with early and late onset infection, Pediatr Infect Dis J, 16, 1997, 370–375, DOI:3.27.2/47c0e9a.
33. Sonawane VB, Mehkarkar NS, Jadhav PB, Gaikwad SU, Kadam NN, Study of interleukin-6 levels in early diagnosis of neonatal sepsis, Int J Res Med Sci, 3, 2015, 41-6, DOI:10.54552320601220150108.
34. Zasloff M, Fighting infections with vitamin D, *Nat Med* ,12,2006,388–390,DOI:10.1038/nm0406-388.
35. Wobke TK, Sorg BL, Steinhilber D, Vitamin D in inflammatory diseases, *Front Physiol,* 5, 2014, 244, DOI:10.33892014.00244.
36. Barker T, Rogers VE, Levy M, Templeton J, Goldfine H, Schneider ED, Dixon BM, Henriksen VT, Weaver LK ,Supplemental vitamin D increases serum cytokines in those with initially low 25-hydroxyvitamin D: a randomized double blind placebo-controlled study, *Cytokine,* 71, 2015, 132–138, DOI:10.1016/j.cyto.2014.09.012.
37. Peterson CA, Heffernan ME, Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH) D concentrations in healthy women, *J Inflamm (Lond)*, 5, 2008, 10, DOI:10.1186/1476-9255-5-10.
38. Sun X, Cao ZB, Zhang Y, Ishimi Y, Tabata I, Higuchi M,Association between serum 25-hydroxyvitamin D and inflammatory cytokines in healthy adults, *Nutrients,* 6, 2014, 221–230, DOI:10.3390/nu6010221.
39. Garcia-Bailo B, Roke K, Mutch DM, El-Sohemy A, Badawi A,Association between circulating ascorbic acid, alpha-tocopherol, 25-hydroxyvitamin D, and plasma cytokine concentrations in young adults: a cross-sectional study, *NutrMetabol,* 9, 2012, 102, DOI:10.1186/1743-7075-9-102.

Source of Support: Nil, **Conflict of Interest:** None.