BMC Pediatrics

RESEARCH

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Vitamin D and gut microbiome in preterm infants

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Abstract

Background The incidence of vitamin D deficiency among pregnant women remains high and is associated with vitamin D deficiency in infants. In normally breastfed infants, *Bifidobacteriaceae* and *Lactobacillaceae* are known to help in maintaining immunotolerance and prevent infection. Vitamin D in the gastrointestinal tract plays a role in determining the composition and function of intestinal bacteria. Preterm infants are vulnerable to intestinal dysbiosis and sepsis due to bacterial translocation. This study aimed to determine the association between vitamin D levels and intestinal dysbiosis.

Methods It was a cohort study conducted in the Neonatal Unit, Cipto Mangunkusumo Hospital, Tertiary hospital in Indonesia, from November 2019 to January 2021. The inclusion criteria in this study were preterm infants with a gestational age of less than 32 weeks or a birth weight of less than 1500 g. Total 25-hydroxyvitamin D (25(OH)D) levels were collected from the umbilical cords of very preterm or very low birth weight infants. A fecal examination was performed on the seventh day of life to assess intestinal bacteria using real-time PCR for four bacterial genera: *Bifidobacteriaceae*, *Lactobacillaceae, Enterobacteriaceae, and Clostridiaceae.*

Results A total of 43 infants were included in this study. Among the subjects, 53.4% had vitamin D deficiency. There was no association identified between vitamin D deficiency and intestinal dysbiosis (RR 0.67; 95% CI (0.15–2.82), *p*-value = 0.531). However, the ratio of *Lactobacillacecae* to *Enterobacteriaceae* was lower in those with vitamin D deficiency.

Conclusion Vitamin D deficiency was not associated with dysbiosis in preterm infants. However, this study found that the ratio of *Lactobacillaceae* to *Enterobacteriaceae* in those with vitamin D deficiency was lower than in those without vitamin D deficiency. Further research is warranted to confirm this finding.

Keywords Dysbiosis, Preterm infants, Vitamin D

^Agus Firmansyah and Saptawati Bardosono are deceased.

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Background

The incidence of maternal vitamin D deficiency is high. Several studies have found that 18–84% of pregnant women have vitamin D deficiency [1]. The prevalence of vitamin D deficiency among first trimester pregnant women in Indonesia was 99.6% [2]. This deficiency is consequentially associated with low vitamin D levels in infants, with approximately 79% of preterm infants suffering from this condition [3]. Very preterm infants (born before 32 weeks) have a higher risk of vitamin D deficiency than late preterm and full-term infants. Notably, Indonesia has a premature birth rate of approximately 15%, making it the fifth highest in the world [4]. However, the association between gestational age and vitamin D status remains unclear [5]. Hospitalized preterm infants are at an increased risk for vitamin D deficiency due to factors such as limited sun exposure during hospital stay, delayed initiation of oral feeding, prolonged fasting, low vitamin D levels in breast milk, and insufficient maternal vitamin D levels [5, 6].

Preterm and very low birth weight infants are vulnerable to intestinal dysbiosis and bacterial translocation through the intestines [7]. Intestinal dysbiosis is defined as an imbalance in microbiota composition, changes in the metabolic activity of the microbiota, or changes in the distribution of the microbiota in the gut [6, 8]. Several factors that may affect this include age, genetics, use of antibiotics, delivery method, nutritional intake (breast milk or formula milk), and postnatal environment (such as neonatal intensive care unit) [9, 10]. Preterm birth causes a delay in gut colonization. Additionally, it results in abnormal pattern of gut microbiota colonization compared with term birth [11]. In normal breastfed infants, *Bifidobacteriaceae* and *Lactobacillaceae*play a role in maintaining immunotolerance and preventing infection. The composition and function of those intestinal bacteria are regulated by the status of the vitamin D receptors [12]. This study aims to determine the association between vitamin D levels and intestinal dysbiosis by comparison of commensals and pathogens ratio.

Methods

This is a prospective cohort study conducted in the Neonatal Unit, Cipto Mangunkusumo Hospital (CMH), Jakarta, from November 2019 to January 2021. The inclusion criteria in this study were preterm infants gestational age < 32 weeks or birth weight of < 1500 g parental or legal guardian consent to participate in the study. The exclusion criteria were infants with lethal congenital abnormalities, suspected of syndrome, and incomplete maternal data. Follow up will be continue until patient discharged or die for a minimum of 7 days.

The dependent variable in this study is intestinal dysbiosis. Independent variable is umbilical cord serum vitamin D level measured as 25-hydroxyvitamin D (25(OH) D). Potential confounding variables for intestinal dysbiosis are maternal vitamin D supplementation, severe preeclampsia, maternal infection, delivery method, gestational age, birth weight, nutritional intake, sepsis, and antibiotic use during hospitalization.

The assessment of total 25-hydroxyvitamin D (25(OH)D)

Determination of total circulating 25(OH)D in umbilical cord was performed using DiaSorin (Liaison®, Saluggia, Italy) analyzer, using a direct competitive chemiluminescence immunoassay (CLIA) method, which was tested in Prodia Child Laboratory. American Academy of Pediatrics (AAP) categorizes the vitamin D status into three categories, deficient (5–15), insufficient (16–20), and sufficient (21–100) [12]. For analysis purposes in this study, vitamin D status was categorized into two categories, deficient ≤ 15 ng/mL and not deficient (insufficient and sufficient) > 15 ng/mL.

Assessment of faecal samples and DNA extraction

Faecal samples were collected from 7-day-old infants (180–220 mg), and were stored at the Gastrohepatology Biomolecular Laboratory of the Department of Child Health, Faculty of Medicine Universitas Indonesia at -20 °C. Samples were added with 1 mL of sterile buffer TN150 (nM Tris–HCl, 150 mM NaCl (pH 8)) and 300 mg of zirconium beads (0.1 mm in diameter) [13].

The real-time PCR examination

All samples were examined at the Gastrohepatology Biomolecular Laboratory of the Department of Child and Health, Faculty of Medicine University Indonesia. Intestinal bacteria from faecal samples were assessed using quantitative real-time PCR (qPCR). The fecal examination was performed on four bacterial genera, i.e., *Enterobacteriaceae* and *Lactobacillaceae*, as well as *Clostridium* and *Bifidobacteriaceae*. The results were presented in copy numbers/200 mg of faeces for each type of bacteria. Quantitative real-time PCR was performed using the ABI 7500 Fast system on MicroAmp Fast Optical 96-Well plates with an optical adhesive film (Biosystems, Foster City, CA, USA). The primer used was working stock primer forward (Aliquor primer). The primer target from the PCR was the 16S rRNA gene from all bacteria, which is a common bacterial structure in human faecal microbiota. All reactions were conducted in a final volume of 20 mL, which contained 1 × Fast SYBR® Green PCR Mastermix (Biosystems), 300 nM each primer, and a 2 mL DNA template. The thermocycling program included polymerase activation at 95°C for 30 s, followed by 40

cycles that consisted of denaturation at 95°C for 10 s, annealing, and elongation at 60°C for 30 s. The fluorescence level was measured after annealing at 60°C. Concentration and purity of the DNA were evaluated using a nanodrop spectrophotometer (Thermo Fisher Scientific), and was then stored in a freezer at -20°C and diluted 10 times from 5×106 to 5×101 genome, calculated using a copy of gene target per genome, which was obtained from genome sequence information (NCBI). All reactions were performed twice in two stages with separate plates. The qPCR results were normalized according to the total target abundance of the 16S rRNA gene, measured with qPCR using primer [13].

Assesment of intestinal dysbiosis

Intestinal dysbiosis describes changes in the intestinal microbiota in relation to a disease [14]. An abundance of *Enterobacteriaceae*is one of the characteristics of dysbiosis [15]. Low numbers of *Bifidobacteriaceae* and high numbers of *Enterobacteriaceae* and *Clostridiaceae* can be a sign of dysbiosis within the first month of life. For analysis purposes, a comparison between *Lactobacillaceae* (representing commensal bacteria) and *Enterobacteriaceae* (representing pathogenic bacteria) was performed, and a ratio of *Lactobacillaceae* to *Enterobacteriaceae* < 1 indicates the presence of intestinal dysbiosis, modified from Paterson et al. [16].

Statistical analysis

All obtained data were recorded using a pre-prepared form. Data entry was performed using the Statistical Package for the Social Sciences (SPSS) program version 20.0 for Windows. Univariate and bivariate analyses were performed. Data which followed normal distribution were presented in mean (± SD), while those which did not follow normal distribution were presented using median (interquartile range). The chi-square test was used to analyze data between two categorical variables. Multivariate analysis was conducted using modified cox proportional hazard regression. Effect size was measured using relative risk (RR) and 95% confidence interval. The significance level for all hypotheses was $p < 0.05$, with a confidence interval of 95%.

Results

This is a secondary analysis as a part of study "The Role of Vitamin in Determining the Morbidity of Very Premature or Very Low Birth Weight Babies: A Review on Regulatory T Cells and Intestinal Dysbiosis". Sample size calculation was address based on that study. The original cohort recruited 119 very preterm and/or very low birth weight infants but a proportion of subject could not undergo fecal examination due to technical issues related to COVID-19 pandemic.

A total of 43 infants of the original cohort were eligible for this analysis. The mean gestational age was 29.56 ± 2.67 weeks, and the mean birth weight was 1,138.21 ± 262.70 g. As many as 53.4% (23 out of 43) subjects were found to have vitamin D deficiency. The infants whose mother received vitamin D supplementation had lower proportion of vitamin D deficiency (40%) than those whose mothers did not (55.2%). There were no differences in the baseline characteristics of subjects in both groups (Table 1).

In the microbiota examination, no *Bifidobacteriaceae* was found from the faecal samples collected. The mean total bacteria were 2,520,300 copy numbers/200 mg of faeces. Detailed bacteria number were 232.26 (± SD) copy numbers/200 mg faeces for *Lactobacillaceae*, 5.25 (± SD) copy numbers/200 mg of faeces for *Clostridiaceae*, and 37,711 (± SD) copy numbers/200 mg of faeces for *Enterobacteriaceae*. A difference in proportion was seen between subjects with vitamin D deficiency and no vitamin D deficiency (Fig. 1). The proportion *Lactobacillaceae* was 67.66% in the group no vitamin D deficiency, whereas it was only 13.16% in the group with vitamin D deficiency.

In our study, 61.1% subjects had intestinal dysbiosis. Subjects with vitamin D deficiency had lower proportion of intestinal dysbiosis although statistically not significant (60.87% vs 70%, p-value = 0.531) (Table 2). There was no association between vitamin D deficiency in newborns and intestinal dysbiosis (RR 0.49; 95% CI (0.10–2.37), p-value = 0.38) after being adjusted by confounding factors (Table 3). On the other hand, we found that sepsis significantly increase the risk of intestinal dysbiosis (OR 8.18 95% CI 1.41–47.23, p-value = 0.01) (Table 3).

Discussion

Vitamin D deficiency was found in 53.4% among very preterm and/or very premature infants in this study. We found no *Bifidobacteriaceae* and only a few *Clostridium* in subject fecal analysis. Dysbiosis, defined as a ratio between *Lactobacillaceae* and *Enterobacteriaceae,* was found in 60.87% subjects and tend to be lower among subjects with vitamin D deficiency. On the other hand, this study did not find association between vitamin D deficiency and intestinal dysbiosis.

There is a high prevalence of vitamin D deficiency in very premature infants. Previous study reported that most of preterm infants had vitamin D insufficiency and half of them were severely vitamin D deficient [4]. In this cohort, vitamin D status was not associated with maternal vitamin D supplementation, pre-eclampsia, and maternal infections ($p > 0.05$). Most of the mothers

Table 1 Baseline characteristics of study participants

Characteristics	Vitamin D deficiency n (%)	No vitamin D deficiency n (%)	*p*-value
Maternal vitamin D supplementation			
No	21 (55)	17 (44.7)	0.43
Yes	2 (40)	3 (60)	
Severe pre-eclampsia			
Yes	10 (58.8)	7 (41.1)	0.57
No	13 (50)	13 (50)	
Maternal infection			
Yes	13 (48.1)	14 (51.8)	0.36
No	10 (62.5)	6 (37.5)	
Delivery method			
Caesarean section	21 (53.8)	18 (46.1)	0.64
Spontaneous delivery	2 (50)	2 (50)	
Gestational age (weeks)	30.00 ± 2.93	29.05 ± 2.30	0.25
Birth weight (grams)	1,147.17 ± 252.14	1,127.90 ± 280.59	0.81
Nutritional intake			
Breast milk	16 (55.1)	13 (44.8)	0.26
Formula milk	7 (50)	7 (50)	
Antibiotic use			
Yes	22 (52.3)	20 (47.6)	0.53
No	1 (100)	0 (0)	
Sepsis			
Yes	13 (52)	12 (48)	0.81
No	10 (55.5)	8 (44.4)	

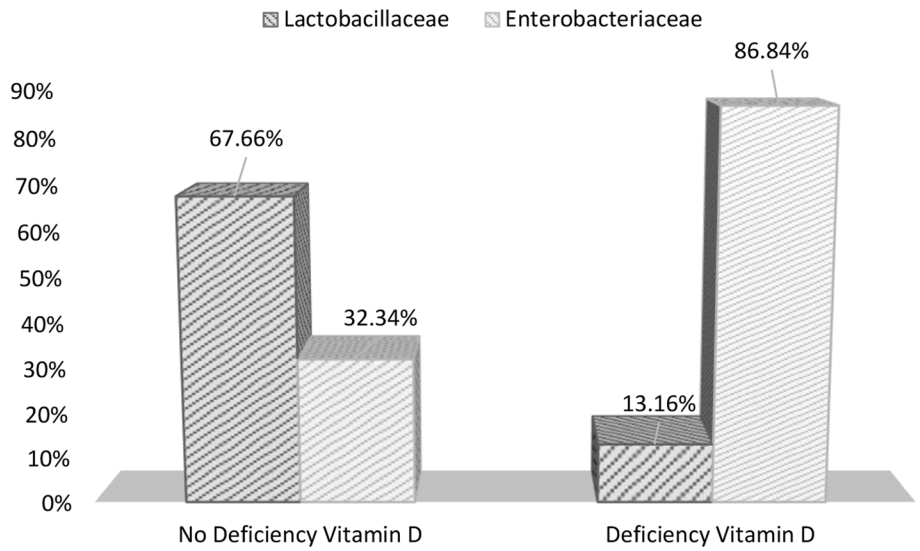

Fig. 1 Show comparison of *Lactobacillaceae/Enterobacteriaceae* between groups with and without vitamin D deficiency

in this study did not consume vitamin D supplementation during pregnancy. In contrast to this study, maternal vitamin D supplementation was positively correlated to maternal serum and cord blood 25(OH)D concentrations [14]. Matejek et al. reported that there is a strong correlation between maternal and cord blood 25(OH)

Table 2 Bivariate analysis between infant vitamin D and Intestinal dysbiosis

Vitamin D status	Intestinal dysbiosis		RR 95%CI	*p*-value
	Yes n (%)	No n(%)		
Deficiency	14(60.87)	9(39.13)	0.67(0.15–2.82)	0.531
No deficiency	14(70.00)	6(30.00)		

Table 3 Association between vitamin D and intestinal dysbiosis after adjusted by confounding factors for intestinal dysbiosis

Confounding factors	RR 95% CI	*p*-value
Maternal vitamin D	1.68(0.15–18.01)	0.66
Gestational Age	1.15(0.84–1.57)	0.37
Infant Vitamin D	0.49(0.10–2.37)	0.38
Nutritional Intake	0.76(0.17–3.32)	0.72
Sepsis	8.18(1.41–47.23)	0.01

D levels indicating inadequate vitamin D stores during pregnancy and the presence of vitamin D deficiency in preterm newborns [15]. Previous studies have indicated that infections can lead to vitamin D deficiency, as infections increase the physiological demand for vitamin D, which plays a crucial role in modulating both innate and adaptive immune responses. Consequently, we hypothesized that maternal infections may lower maternal vitamin D levels, thereby impacting transplacental transfer and potentially resulting in vitamin D deficiency in neonates [16, 17].

This study demonstrated that there were no associations between gestational age or birth weight and vitamin D status ($p > 0.05$), which was similar to the findings of several previous studies [15, 18]. There were no significant differences in serum 25(OH)D concentrations or the incidence of severe vitamin D deficiency between early, moderate, and late preterm infants. Therefore, it was hypothesized that vitamin D status may be related to other factors such as maternal serum levels during pregnancy. No endogenous production of 25(OH)D occurs in the fetus, and hence its levels depend on transplacental transfer [19].

Intestinal dysbiosis is defined as low microbiota diversity, lack of commensal microbiota, and excessive growth of pathogenic microbiota [6, 20]. Intestinal dysbiosis describes changes in the intestinal microbiota in relation to a disease [20]. In general, low *Bifidobacteriaceae* and high *Enterobacteriaceae* and *Clostridiaceae*proportion can be a sign of dysbiosis within the first month of life. This pattern is often found in various conditions, including premature infants, full-term infants requiring long-term treatment or antibiotics, and infants with good clinical conditions with an increased risk for dysbiosis-associated diseases [20]. Based on the definition above, we found all of the subjects in our study were dysbiotic, characterized by low number of *Bifidobacteriaceae* and high number of *Enterobacteriaceae*.

The microbiota composition in newborns can be affected by many factors from pregnancy to birth. Previously, the intestines of an infant were thought to be sterile before birth. However, with time, several studies identified the presence of bacteria inside the meconium, amniotic fluid, and placenta [7]. This finding opened a possibility that intrauterine factors can also affect the composition of intestinal microbiota in newborns. One mechanism for the presence of microbiota in the intestine of newborns is through the process of ingesting amniotic fluid. The diversity and abundance of microbiota in newborns increases along with their development.

Vitamin D deficiency causes a decrease in microbiota clearance in intestine, decreased expression of intestinal epithelial tight junctions, and increased Th1 cell-mediated inflammation [21]. Intestinal microbiota imbalance can be influenced by the number of vitamin D receptors [12]. Premature and/or very low birth weight infants are thought to be more susceptible to intestinal dysbiosis [7].

An interesting trend was also observed in this study regarding the ratio between commensal and pathogenic bacteria in preterm neonates with vitamin D deficiency, with a lower proportion of commensal bacteria compared to pathogenic bacteria (13.16%: 86.84%). In contrast, opposite results were observed in the group no vitamin D deficiency in preterm neonates (67.66%: 32.34%) (Fig. 1). Low levels of vitamin D in premature infants can cause an imbalance in gastrointestinal microbiota, leading to intestinal dysbiosis [12, 21].

Intestinal microbiota also played an important role for the development of the immune system and immune responses following birth [22]. Vitamin D modulates the immune system by regulating one of its main roles (antimicrobial peptide, AMP). The antimicrobial peptide has various functions aside from its microbiocidal activity, which include chemotaxis of inflammatory immune cells. This means that the level of AMP expression will affect the relative composition of intestinal microbiota. The role of vitamin D in regulating AMP production shows that vitamin D status can affect intestinal microbiota composition [23].

A limitation of this study is that it did not perform microbiota sequencing to better assess intestinal dysbiosis through higher analytical sensitivity, greater resolution of genomic variants, and more data from smaller DNA amounts. We realize that the number of the sample was

small but yet valuable for the study. This was the first study in Indonesia which correlated vitamin D and neonatal dysbiosis in premature infant.

Conclusion

Vitamin D was not associated with dysbiosis in premature infants. However, this study found that the ratio of *Lactobacillaceae* to *Enterobacteriaceae* in those with vitamin D deficiency was lower than in those without vitamin D deficiency. Further research is warranted to confirm this finding.

Abbreviations

CMH	Cipto mangunkusumo hospital
AMP	Antimicrobial peptide
SPSS	Statistical package for the social sciences
RR	Relative risk
SD	Standard deviation

Acknowledgements

The authors would like to thank the doctors, nurses, and all team members at the Neonatal Unit of Cipto Mangunkusumo Hospital (CMH), Jakarta for their participation in this study.

Authors' contributions

(PMTM): Conceptualization, Methodology, Validation, Format analysis, Investigation, Writing-original draft, Writing-review and editing. (AF): Conceptualization, Methodology, Validation, Supervision, Writing-original draft. (RR): Conceptualization. (YP): Conceptualization. (SB): Methodology, Format analysis. (SGM): Methodology, Validation, Writing-review and editing, Supervision. (ZM): Investigation. (IST): Investigation. (TY): Investigation. (MY): Investigation, Writing-original draft, Writing-review and editing.

Funding

The authors would like to thank Universitas Indonesia for funding this study through the PUTI Grant, with the following contract number: NKB-3517/UN2.RST/HKP.05.00/2020.

Availability of data and materials

The data are available from the corresponding author on reasonable request. Medical records are available in the Archive of Neonatal Unit, Cipto Mangunkusumo Hospital.

Declarations

Ethics approval and consent to participate

This study obtained ethical clearance from the Ethical Committee of the Faculty of Medicine, Universitas Indonesia with the following registration number: 617/UN2.F1/ETIK/PPM.00.02/2019. Informed consent to participate was obtained from the parents or legal guardians, as our study involved a population of children under the age of 16.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 7 June 2024 Accepted: 4 September 2024
Published online: 16 September 2024

References

1. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. J Clin Endocrinol Metab. 2007;92(9):3517–22.
2. Wibowo N, Bardosono S, Irwinda R, Syafitri I, Putri A, Prameswari N. Assessment of the nutrient intake and micronutrient status in the first trimester of pregnant women in Jakarta. Med J Indonesia. 2017;26:109.
3. Boskabadi H, Zakerihamidi M, Faramarzi R. The vitamin D level in umbilical cord blood in premature infants with or without intra-ventricular hemorrhage: a cross-sectional study. Int J Reprod Biomed. 2018;16(7):429–34.
4. Marsubrin PMT, Ibrahim NAA, Dilmy MAF, Ariani Y, Wiweko B, Irwinda R, et al. Determinants of prematurity in urban Indonesia: a meta-analysis. J Perinat Med. 2024;52(3):270–82.
5. Park SH, Lee GM, Moon JE, Kim HM. Severe vitamin D deficiency in preterm infants: maternal and neonatal clinical features. Korean J Pediatr. 2015;58(11):427–33.
6. Groer MW, Luciano AA, Dishaw LJ, Ashmeade TL, Miller E, Gilbert JA. Development of the preterm infant gut microbiome: a research priority. Microbiome. 2014;2(1): 38.
7. Yang I, Corwin EJ, Brennan PA, Jordan S, Murphy JR, Dunlop A. The infant microbiome: implications for infant health and neurocognitive development. Nurs Res. 2016;65(1):76–88.
8. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol. 2014;16(7):1024–33.
9. Qari SA, Alsufyani AA, Muathin SH, El Margoushy NM. Prevalence of respiratory distress syndrome in neonates. Egypt J Hosp Med. 2018;70(2):257–64.
10. Lykkedegn S, Sorensen GL, Beck-Nielsen SS, Christesen HT. The impact of vitamin D on fetal and neonatal lung maturation. A systematic review. Am J Physiol Lung Cell Mol Physiol. 2015;308(7):L587-602.
11. Moles L, Gómez M, Heilig H, Bustos G, Fuentes S, de Vos W, et al. Bacterial diversity in meconium of preterm neonates and evolution of their fecal microbiota during the first month of life. PLoS ONE. 2013;8(6):e66986.
12. Jin D, Wu S, Zhang YG, Lu R, Xia Y, Dong H, et al. Lack of vitamin D receptor causes dysbiosis and changes the functions of the murine intestinal microbiome. Clin Ther. 2015;37(5):996-e10097.
13. Oswari H, Prayitno L, Dwipoerwantoro PG, Firmansyah A, Makrides M, Lawley B, et al. Comparison of stool microbiota compositions, stool alpha1-antitrypsin and calprotectin concentrations, and diarrhoeal morbidity of Indonesian infants fed breast milk or probiotic/prebiotic-supplemented formula. J Paediatr Child Health. 2013;49(12):1032–9.
14. United Nations International Children's Emergency Fund. Maternal and newborn health disparities. Unicef Indonesia; 2023.
15. Matejek T, Navratilova M, Zaloudkova L, Malakova J, Maly J, Skalova S, et al. Vitamin D status of very low birth weight infants at birth and the effects of generally recommended supplementation on their vitamin D levels at discharge. J Matern Fetal Neonatal Med. 2020;33(22):3784–90.
16. Aranow C. Vitamin D and the immune system. J Investig Med. 2011;59(6):881–6.
17. Taha R, Abureesh S, Alghamdi S, Hassan RY, Cheikh MM, Bagabir RA, et al. The relationship between vitamin D and infections including COVID-19: any hopes? Int J Gen Med. 2021;14:3849–70.
18. Matejek T, Zemankova J, Malakova J, Cermakova E, Skalova S, Palicka V. Severe vitamin D deficiency in preterm infants: possibly no association with clinical outcomes? J Matern Fetal Neonatal Med. 2022;35(8):1562–70.
19. Merewood A, Mehta SD, Chen TC, Bauchner H, Holick MF. Association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab. 2009;94(3):940–5.
20. Litvak Y, Byndloss MX, Tsolis RM, Bäumler AJ. Dysbiotic proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr Opin Microbiol. 2017;39:1–6.
21. Cetinkaya M, Erener-Ercan T, Kalayci-Oral T, Babayiğit A, Cebeci B, Semerci SY, et al. Maternal/neonatal vitamin D deficiency: a new risk factor for necrotizing enterocolitis in preterm infants? J Perinatol. 2017;37(6):673–8.
22. Kaplan JL, Shi HN, Walker WA. The role of microbes in developmental immunologic programming. Pediatr Res. 2011;69(6):465–72.
23. Talsness CE, Penders J, Jansen EHJM, Damoiseaux J, Thijs C, Mommers M. Influence of vitamin D on key bacterial taxa in infant microbiota in the KOALA birth cohort study. PLoS ONE. 2017;12(11):e0188011.

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