Research Article

Increased Levels of Umbilical Cord Blood Interleukin-6 (IL-6) and Serum C-Reactive Protein (CRP) in Premature Infants of Vitamin D Deficient Mothers

Peningkatan Kadar Interleukin-6 (IL-6) Darah Tali Pusat dan Serum C-Reactive Protein (CRP) pada Bayi Prematur dari Ibu dengan Defisiensi Vitamin D

Yuyun Lisnawati,[1] Yesy Marianna,[1] Rinawati Rohsiswatmo[3]

[1]Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Indonesia
Persahabatan Hospital
[2]Department of Pediatric
Faculty of Medicine Universitas Indonesia
Dr. Cipto Mangunkusumo General Hospital
Jakarta

Abstract

Objective: Increased levels of inflammatory factors in newborns are often associated with lower maternal vitamin D levels. This study aimed to find out the relationship between maternal and umbilical cord vitamin D serum levels on umbilical cord Interleukin-6 (IL-6) and serum C-Reactive Protein (CRP) levels in premature infants.

Methods: The study was an observational analytic, cross-sectional design in mothers who underwent preterm birth at 28-34 weeks' gestation due to premature rupture of membranes (PROM) and their infants at Dr. Cipto Mangunkusumo General Hospital (RSCM), Jakarta and Persahabatan General Hospital, Jakarta, from January 2017 to August 2018. Levels of serum vitamin D of the maternal and umbilical cord, umbilical cord IL-6 and serum CRP in premature infants were recorded. Vitamin D level was divided into deficiency (<10 ng/mL), insufficiency (10–29 ng/mL), and normal (≥30 ng/mL) groups. The relationship of vitamin D levels with IL-6 and CRP was carried out using Kruskal Wallis test.

Results: A total of 70 subjects met the research criteria. Umbilical cord IL-6 and serum CRP levels in premature infants of vitamin D deficient mothers were higher (20.31 pg/mL and 0.50 mg/L) compared to insufficient (3.34 pg/mL and 0.45 mg/L) and normal mothers (3.29 pg/mL and 0.30 mg/L), although not statistically significant (IL-6 p = 0.665, CRP p = 0.89). Referring to the umbilical cord blood vitamin D levels, the results were different and not as expected, in which the umbilical cord IL-6 and serum CRP levels of preterm infants in the deficiency (3.76 pg/mL and 0.35 mg/L) and insufficiency (3.37 pg/mL and 0.40 mg/L) groups were lower (IL-6) and not different (CRP) than the normal group (9.41 pg/mL and 0.40 mg/L).

Conclusions: There were atendency for an increase in umbbilical cord IL-6 and serum CRP level in premature infants of Vitamin D deficient mother although these were not statistically significant. Based on the levels of vitamin D umbilical cord blood, the CRP levels in the serum of premature infants were not different, while the IL-6 levels in the deficiency and insufficiency group were lower than in the normal group.

Keywords: CRP, IL-6, maternal vitamin D, umbilical cord vitamin D.

Abstrak

Tujuan: *Peningkatan kadar faktor inflamasi pada bayi baru lahir sering dikaitkan dengan rendahnya kadar vitamin D ibu. Penelitian ini bertujuan untuk mengetahui hubungan kadar serum vitamin D ibu dan tali pusat, dengan kadar IL-6 tali pusat dan serum C-Reactive Protein (CRP) bayi prematur.*

Metode: *Studi observasional analitik dengan desain potong lintang pada subjek ibu yang mengalami kelahiran prematur di usia 28–34 minggu kehamilan disebabkan ketuban pecah dan bayi yang dilahirkannya, di Rumah Sakit Umum Pusat Nasional dr. Cipto Mangunkusumo (RSCM) dan Rumah Sakit Umum Pusat Persahabatan, Jakarta, pada bulan Januari 2017 sampai Agustus 2018. Variabel data adalah kadar serum vitamin D ibu dan tali pusat, kadar serum IL-6 tali pusat dan kadar CRP darah bayi. Kadar vitamin D (25(OH)D) dibagi menjadi defisiensi (<10 ng/mL), insufisiensi (10–29 ng/mL) dan normal (≥30 ng/mL) dan dicari hubungannya dengan kadar IL-6 tali pusat dan serum CRP bayi prematur, menggunakan uji Kruskal Wallis.*

Hasil: *Sebanyak 70 subjek telah memenuhi kriteria penelitian. Kadar IL-6 tali pusat dan serum CRP bayi prematur dari kelompok ibu defisiensi vitamin D (20,31 pg/ml dan 0,50 mg/L) lebih tinggi dibandingkan kelompok ibu insufisiensi vitamin D (3,34 pg/mL dan 0,45 mg/L) maupun kelompok ibu normal vitamin D (3,29 pg/mL dan 0,30 mg/L) tetapi perbedaan tersebut tidak bermakna (IL-6 p=0,665 dan CRP p = 0,899). Mengacu pada kadar vitamin D darah tali pusat didapatkan hasil yang berbeda dan tidak sesuai harapan, dimana tali pusat IL-6 dan serum CRP bayi prematur mengalami defisiensi (3,76 pg / mL dan 0,35 mg / L) dan insufisiensi. (3,37 pg / mL dan 0,40 mg / L) kelompok lebih rendah (IL-6) dan tidak berbeda (CRP) dibandingkan kelompok normal (9,41 pg / mL dan 0,40 mg / L).*

Kesimpulan: *Didapat kecenderungan peningkatan kadar IL-6 darah tali pusat dan serum CRP bayi prematur dari ibu dengan defisiensi kadar vitamin D walaupun secara statistik tidak signifikan. Berdasarkan kelompok vitamin D darah tali pusat, kadar CRP serum bayi prematur tidak berbeda, sedangkan kadar IL-6 pada kelompok defisiensi dan insufisiensi lebih rendah dibandingkan pada kelompok normal.*

Kata kunci: *CRP, IL-6, vitamin D ibu, vitamin D tali pusat.*

Correspondence author. Yuyun Lisnawati. yulisna.er@gmail.com

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INTRODUCTION

Preterm birth is one of the most prominent causes of perinatal death and long term morbidity.[1,2] Several complications in form of growth retardation, airway problems, and digestive tract disorders are significantly higher in premature infants.[3] Epidemiologically, prevalence of preterm birth differs in every country, ranging from 5-18% of live births. Prevention of premature birth is one of the global priority in medical fields until today.[2]

The main causes of preterm birth are still a matter of debate. One of the theories states that inflammatory mediator activation would excite myometrium and induce contraction.[4] Previous study has shown that increase in inflammatory cytokines such as IL-6 and IL-8 was noted in pregnant women experiencing preterm birth.[4] However, most of the cases remain to be idiopathic, raising a hypothesis of subclinical response to an inflammation on mother and fetus.5

Premature Rupture of Membranes (PROM) is associated with subclinical intrauterine infection in preterm birth. Chorioamnionitis as a complication of PROM would increase the level of proinflammatory cytokines in amniotic fluid, umbilical cord, and maternal serum. Those proinflammatory cytokines would circulate in maternal blood circulation, inducing the synthesis of C-reactive protein (CRP) in hepatocyte and leukocyte in bone marrow.[5]

Synthesis of proinflammatory cytokines is strongly associated with immune response in the body. One of the contributing factors in modulating immune response is nutrition, one of which is vitamin D *(25-dihydroxyvitamin D3).* Vitamin D in pregnancy in its active form *1,25-dihydroxyvitamin* D3 [$1,25(OH)_2D_3$] would affect innate response immune through trophoblast, lowering inflammation and oxidative stress marker.[6] It would also induce cathelicidin in various tissues to reduce bacterial infection, including in the placenta.[6] Lower level of maternal vitamin D has been reported to stimulate acute phase response, increasing CRP, hemostatic factors, and proinflammatory concentration in both mothers and newborns.[7]

Vitamin D also plays a role in regulating the adaptive immune response. Vitamin D in the umbilical cord is correlated with antimicrobial substance production and inflammatory response induced by Toll Like Receptor (TLR). Escalation in vitamin D level would modulate and decrease the level of TLR 2, TLR 4, and TLR 9, thus decrease the secretion of IL-6 as one of the inflammatory markers in circulation.[8,9]

METHODS

This was a cross-sectional study determined to the relationship of serum maternal and umbilical cord vitamin D levels on umbilical cord IL-6 and serum CRP levels in premature infants. It was performed on August 2019 to July 2020, at RSCM and Persahabatan General Hospital, Jakarta, Indonesia.

Subjects were pregnant mothers at 28–34 weeks of gestation undergoing preterm birth preceded by PROM and their premature infants. Infants with birth weight >2.500 grams, having lethal congenital defects, stillbirth, or having intrauterine infection were excluded from the study. Pregnant women with systemic disease such as diabetes, thyroid disease, vascular disease, chronic hypertension, and liver disease were also excluded from the study. Maternal peripheral blood samples of 5 cc were taken at delivery to check levels of vitamin D. Five cc of umbilical cord blood was taken to check the levels of 25(OH)D and IL-6. Vitamin D serum levels were examined in the form of 25(OH)D, measured by the HPLC-MS (High-Performance Liquid Chromatography Mass Spectrometer) method. Serum IL-6 levels were measured using the ELISA method (Enzyme-Linked Immunosorbent Assay). Serum CRP was collected within 48 hours of the infant's life and examined using an immunoturbidimetric CRP assay. Subjects were taken by consecutive sampling method.

The ethical clearance was approved by the Research Ethics Committee Faculty of Medicine, Universitas Indonesia and Persahabatan Hospital Ethics Committee with ethical clearance number with number KET 798/UN2.F1/ETIK/PPM.00.02/2019 and LB.02.01/1.4.6/343/2019. All patients who were included in this study had given their informed consent prior to their inclusion in the study.

Collected data were then analyzed using SPSS for Macintosh ver. 20. Sociodemographic characteristics of subjects were analyzed descriptively. Vitamin D levels were categorized as deficiency (<10 ng/mL), insufficiency (10–29 ng/mL), and normal (≥30 ng/mL). The data was then analyzed for its relationship with umbilical cord IL-6 and serum CRP premature infants levels used Kruskal Wallis test.

RESULTS

During the period, a total of 70 subjects met the inclusion criteria and had been further analyzed. The sociodemographic characteristics of the subjects are shown in table 1.

Table 1. Sociodemographic and Clinical Characteristics of Subjects

Characteristics	n = 70
Maternal age (years)	30.39 + 6.74
< 35	45 (64.3)
≥ 35	25 (35.7)
Education	
≤ High school	54 (77.1)
> High school	16 (22.9)
Occupation	
Employee	18 (25.7)
Housewife	52 (74.3)
Parity	
1st	32 (45.7)
2nd	13 (18.6)
Multiple	25 (35.7)
Gestational age (weeks)	31.59 + 1.97
28 – 31+6	33 (47.1)
32 – 34+6	37 (52.9)
PROM duration (days)	
1 – 3	55 (78.6)
4 – 6	10 (14.3)
>7	5 (7.1)
Birth weight (gram)	1753.29 + 369.87
1000 – 1500	18 (25.7)
1501 – 2000	35 (50.0)
2001 – 2500	17 (24.3)

Data distribution of serum 25(OH)D, IL-6 and CRP levels can be seen in table 2. Relationship between 25(OH)D level with inflammatory markers can be found in Table 3 (maternal) and Table 4 (umbilical cords).

Table 2. Data Distribution of Serum 25(OH)D, Interleukin-6 and C-Reactive Protein

Laboratorium Parameters	Median (min – max)
25(OH) D maternal serum (ng/mL)	23.07 (3.32 – 68.53)
25(OH) D umbilical cord (ng/mL)	13.05 (2.25 – 53.35)
IL-6 umbilical cord (pg/mL)	3.42 (0.45 – 35.72)
CRP infant (mg/L)	0.40 (0.02 – 35)

Table 3. Relationship between Maternal 25(OH)D, Interleukin-6 and C-Reactive Protein

Markers	Maternal 25(OH)D (ng/mL)			*P-value*
	Normal (n=22)	Insufficiency (n=42)	Deficiency (n=6)	
IL-6 umbilical cord (pg/mL)	3.29 (0.63 – 35.72)	3.34 (0.45 – 35.14)	20.31 (1.12 – 33.40)	0.665
CRP infants (mg/L)	0.30 (0.10 – 3.00)	0.45 (0.02 – 35.00)	0.50 (0.10 – 6.40)	0.899

Table 4. Relationship between Umbilical Cord 25(OH)D, Interleukin-6 and C-Reactive Protein

Markers	Umbilical cord 25(OH)D (ng/mL)			*P-value*
	Normal (n=9)	Insufficiency (n=41)	Deficiency (n=20)	
IL-6 (pg/mL)	9.41 (1.26 – 32.97)	3.37 (0.45 – 35.72)	3.76 (1.08 –35.02)	0.758
CRP (mg/L)	0.40 (0.10 – 2.50)	0.40 (0.02 – 10.30)	0.35 (0.03 –35.00)	0.815

DISCUSSION

This study found that maternal vitamin D or 25(OH)D levels generally showed insufficiency with a median of 23.07 (3.32–68.53) ng/mL. These results were similar to that of many studies that found vitamin D levels in pregnant women were generally insufficient. This study also found that the median umbilical cord 25(OH)D level was 13.05 (2.25–53.35) ng/mL. Study with large subjects, showed a median umbilical cord vitamin D 39.43 nmol/l or equal to 15.8 ng/mL, similar with the result of this study where umbilical cord 25(OH)D levels were insufficient.[10]

Based on review articles and previous studies, it was stated that vitamin D plays a role in inhibiting the production of the cytokine IL-6. Low vitamin D levels may not reduce the release of inflammatory cytokines. In this study, it was found that there was a tendency for umbilical cord IL-6 and infant CRP serum levels to be higher in the group of vitamin D deficient mothers. This difference

was seen, especially in IL-6 levels. However, the difference in levels of umbilical cord IL-6 and infant CRP serum based on maternal vitamin D category was not statistically significant.

In one study reported comparisons of maternal serum vitamin D, serum CRP and IL-6 levels taken at 72 hours of life in infants without and with early clinical onset sepsis.[11] The levels of maternal vitamin D were 36.047 ± 1.243 ng/mL vs 22.3 ± 5.047 ng/mL, CRP levels were 5.1 ± 3.6 mg/dL vs 14.78 ± 9.3 mg/dL, and IL-6 levels were 24.71 ± 35.46 pg/mL vs 198.074 ± 59.58 pg/mL, respectively. It appears that at higher maternal vitamin D levels, CRP and IL-6 levels were found to be lower.[11] This is similar to the results in this study, where at higher maternal vitamin D levels, the infant's CRP and IL-6 umbilical cord levels were lower, in addition to different IL-6 sample origins.

This study found that at maternal general insufficient 25(OH)D levels of 23.07 (3.32–68.53) ng/mL, serum umbilical cord IL-6 levels showed a median of 3.42 (0.45–35.72) pg/mL. In one study in China regarding vitamin D and serum IL-6 as risk factors for tubal infertility, it was found that at a insufficient 25(OH)D level of 19.4 ng/mL (15.8–22.7 ng/mL), the serum IL-6 levels was 5.3 (4–7.5) pg/mL.[12] At first glance the results look similar. We have not been able to compare the results with previous studies with the same sample, so differences in sample types should be a concern.

At insufficient levels of 25(OH)D, in this study and other study[11], it were found that serum CRP levels were 0.40 (0.02–35) mg/L and 6.71 (SD = 3.07) mg/L. The different results may be due to differences in sample types, our study used serum CRP taken within 48 hours of the infant's life whereas another study[11] used umbilical cord CRP samples.

Several studies with similar variables but in different specimens may be able to compare the association of vitamin D with inflammatory factors. One study reported a significant negative correlation between maternal serum vitamin D and CRP levels in infants with early-onset sepsis ($r = -0.75$, $p < 0.001$).[13] A significant negative correlation was also found between neonatal vitamin D and IL-6 levels taken at 72 hours of infant life with early-onset sepsis ($r = -0.923$, $p < 0.001$).[11] Another study in healthy women aged 25 to 82 years reported that there appears to be a slight trend toward an inverse association between 25(OH)D levels and serum IL-6 ($p = 0.0909$).[14] These results indicated that the higher the maternal vitamin D levels, the lower the IL-6 and CRP levels are expected.

Other studies have shown different results on the association of vitamin D with serum IL-6 and CRP. There was no significant association between serum vitamin D and CRP levels in asymptomatic adults.[15] There was no significant difference in serum IL-6 levels with vitamin D levels by category in patients with rheumatoid arthritis.[16]

In contrast to maternal vitamin D, unexpected results were obtained for umbilical cord vitamin D. The infant's high umbilical cord IL-6 and CRP levels were seen at normal vitamin D levels. Based on the theory, the better the vitamin D level, it is expected that IL-6 and CRP levels will tend to decrease. The imbalance in the number of umbilical cord vitamin D samples based on the level category, may affect the statistical results and be one of the weaknesses of this study. Future studies with larger and balance samples will likely be able to reach theoretically and clinically relevant conclusions.

CONCLUSION

There were atendency for an increase in umbbilical cord IL-6 and serum CRP level in premature infants of Vitamin D deficient mother although these were not statistically significant. Based on the levels of vitamin D umbilical cord blood, the CRP levels in the serum of premature infants were not different, while the IL-6 levels in the deficiency and insufficiency group were lower than in the normal group.

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CONFLICT of INTEREST

Authors declare that there is no conflict of interest in this study.

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