Research Article

Placental Vitamin D, Oxidative Stress, and Senescence Markers in Spontaneous Preterm Birth: A Comparative Cross-Sectional Study

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Abstract

Objective: To evaluate the associations between vitamin D metabolism markers and biomarkers of oxidative stress and placental senescence among women with spontaneous preterm labor compared with those with term labor.

Methods: We conducted a comparative cross-sectional study between 2017 and 2019 in two hospitals in Jakarta, Indonesia. Maternal serum and placental samples were collected from women with term labor and spontaneous preterm labor. Markers of the vitamin D pathway—25-hydroxyvitamin D (25[OH]D), 1,25-dihydroxyvitamin D_3 (1,25$[OH]_2D_3$), vitamin D receptor (VDR), and CYP27B1—along with oxidative stress (8-hydroxy-2--deoxyguanosine [8-OHdG]) and placental senescence markers (GLB1 and HMGB), were measured using ELISA, LC–MS/MS, and ICP–MS. Between-group comparisons were performed using parametric or nonparametric tests, as appropriate, and correlations were assessed using Pearson's or Spearman's correlation coefficients.

Results: A total of 67 women were included (term labor, n = 34; spontaneous preterm labor, n = 33), and both groups were vitamin D deficient. Placental 1,25$(OH)_2D_3$ levels were significantly lower in the preterm group than in the term group (4.58 ± 2.90 vs 5.57 ± 3.50 pg/ng, p = 0.037). Placental VDR levels also differed significantly between groups {21.70 (6.06–73.40) vs 16.48 (1.87–74.67), p = 0.041}. Across all participants, 8-OHdG and placental senescence markers were negatively correlated with placental 25(OH)D and 1,25$(OH)_2D_3$ levels and positively correlated with placental CYP27B1 and VDR expression.

Conclusion: In this comparative cross-sectional cohort, placental vitamin D metabolites were associated with lower levels of oxidative stress and placental senescence biomarkers, whereas VDR and CYP27B1 showed positive associations with these biomarkers. These patterns may reflect compensatory regulatory mechanisms in the context of maternal vitamin D deficiency. These findings are hypothesis-generating and warrant confirmation in prospective studies and mechanistic investigations.

Keywords: oxidative stress, placental senescence, spontaneous preterm labor, vitamin D.

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INTRODUCTION

Prematurity is the leading cause of neonatal mortality and ranks as the second most common cause of death among children under five years of age. The World Health Organization (WHO) reported in 2008 that approximately 15 million babies are born preterm each year worldwide, with various short- and long-term complications. Indonesia ranks fifth globally in the number of preterm births.[1]

Preterm birth is multifactorial in nature. Several risk factors for preterm birth have been identified, including genetic predisposition, infection or inflammation, oxidative stress, excessive uterine stretching, cervical abnormalities, progesterone (P4) resistance, and cellular senescence.[2,3]

Oxidative stress and cellular senescence

are closely interconnected. Oxidative stress is defined as an imbalance between oxidants and antioxidants, resulting in excessive oxidant production that causes molecular damage through free radical activity. One commonly used biomarker of oxidative stress is 8-hydroxy-2-deoxyguanosine (8-OHdG), which reflects oxidative DNA damage.[4,5]

Cellular senescence, particularly decidual senescence, is characterized by the permanent arrest of cell proliferation at the G1 phase of the cell cycle. Senescence may be triggered by telomere dysfunction, DNA damage, strong mitogenic signaling, epigenomic disruptions, and the overexpression of certain oncogenes. Senescent cells exhibit sterile inflammation and release senescence-associated secretory phenotype (SASP) factors and damage-associated molecular patterns (DAMPs), including high-mobility group box 1 (HMGB1) and senescence-associated β-galactosidase (SA-β-gal), which is encoded by the β-galactosidase gene (GLB1).[5-7]

Nutrition plays an important role in preventing preterm birth and involves both macronutrients and micronutrients. In recent years, vitamin D has gained attention for its role in modulating immune activity. During pregnancy, vitamin D regulates the maternal–fetal immune response by enhancing endogenous antioxidant activity, promoting trophoblast invasion, preventing excessive cell death, and maintaining myometrial relaxation. Unfortunately, approximately 99% of pregnant women in Indonesia experience vitamin D deficiency. To date, no studies in Indonesia have evaluated the association between preterm birth and vitamin D status. Therefore, this study aims to determine the association between vitamin D concentrations and senescence biomarkers in term and preterm births.[8-10]

METHODS

Study design and setting

This comparative cross-sectional study evaluated placental biomarkers of senescence (GLB1, HMGB1, and telomere-related measurements [specify method]), oxidative stress (8-hydroxy-2-deoxyguanosine [8-OHdG]), and vitamin D metabolism (25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], vitamin D receptor [VDR], and CYP27B1) among women with spontaneous preterm labor and those delivering at term. The study was conducted at RSUPN Dr. Cipto Mangunkusumo and RS Budi Kemuliaan, Jakarta, Indonesia, from January 2017 to August 2019. Written informed consent was obtained from all participants. Given the modest sample size, the study was designed primarily to detect moderate between-group differences and to generate hypotheses regarding biomarker associations.

Participants and clinical definitions

Participants were classified into two groups; spontaneous preterm labor, defined as delivery at 26–36 weeks' gestation, and term labor (control group), defined as delivery at ≥37 weeks' gestation by vaginal delivery or cesarean section after the onset of labor. Exclusion criteria were applied to reduce clinical heterogeneity and included multiple gestation, intrauterine growth restriction (IUGR), congenital anomalies, preterm premature rupture of membranes (PPROM), and maternal comorbidities.

Specimen collection and placental sampling

Maternal venous blood samples were collected for the measurement of serum 25(OH)D concentrations. Placental tissue was obtained immediately after delivery using a standardized sampling protocol. Full-thickness biopsies were collected from the placental parenchyma while avoiding infarcted, calcified, hemorrhagic areas, as well as the membranes and decidua. Samples were placed in phosphate-buffered saline (PBS), stored at 4°C for up to 24 hours, and subsequently transferred to −80°C until laboratory analysis.

Laboratory measurements

Placental concentrations of GLB1, HMGB1, 8-OHdG, VDR, and CYP27B1 were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturers' instructions. Placental concentrations of 25(OH)D and 1,25$(OH)_2D_3$ were measured using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with an Agilent 1290 LC system coupled to an Agilent Triple Quad 6460 mass spectrometer.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 20. Continuous variables

were assessed for normality and are presented as mean ± Standard Deviation (SD) or median (interquartile range [IQR]), as appropriate. Between-group differences were evaluated using independent-samples t-tests or Mann–Whitney U tests, while categorical variables were compared using χ^2 or Fisher's exact tests. Associations between vitamin D pathway markers and oxidative stress or senescence biomarkers were assessed using Pearson's or Spearman's correlation coefficients, depending on data distribution.

Table 1. Characteristics of subjects

Characteristics	Control (n=34)	Preterm (n=33)	*P-value*
Maternal age (years)	29.59 ± 5.79	27.79 ± 7.36	0.268
Gestational age (weeks)	39.44 ± 1.13	32.53 ± 2.57	<0.001
BMI (kg/m^2)	23.75 (16.6-24.8)	21.70 (14.4-24.6)	0.129
History of Preterm birth			
No	32	31	0.070
Yes	2	2	
Birth Weight (grams)	3176.71 ± 400.07	1884.56 ± 542.59	<0.001

A total of 34 subjects in the control group and 33 subjects in the preterm group participated. Table 1 shows the characteristics of the subjects, with mean gestational age of 39.44 ± 1.13 weeks in control group and 32.53 ± 2.57 weeks in preterm group (p <0.001). There was no difference in body mass index (BMI) and history of preterm birth. Preterm group had significantly lower birth weight of 1884.56 ± 542.59 g, compared to 3176.71 ± 400.07 in control group (p <0.001).

Table 2. Concentrations of Vitamin D and Senescence Markers in Term and Preterm Groups

Markers	Control	Preterm	*P-value*
Maternal Serum			0.977
25(OH)D (ng/ml)	16.58±6.80	16.86±10.96	
Placenta			0.457
25(OH)D (ng/g)	30.43±37.9	28.89±27.05	0.520
CYP27B1 (pg/mg)	7.86±3.87	7.64±3.22	0.037
1,25(OH)2D3 (pg/mg)	5.57±3.50	4.58±2.90	0.041
VDR tissue (pg/mg)	16.48 (1.87-74.67)	21.70 (6.06-73.4)	
Senescence Markers			0.144
8-OHdG (pg/mg)	0.98 (0.55-9.24)	0.69 (0.48-8.23)	0.936
GLB1 (pg/mg)	1568.10±759.71	1546.70±659.84	0.085
HMGB (pg/mg)	135.50±61.88	156.15±49.05	

Table 2 shows the concentration of vitamin D derivatives, receptors, and activating enzymes, as well as senescence markers in control and preterm groups. Both groups had low mean serum 25(OH)D level, which was defined under 30 ng/ml according to Institute of Medicine. Preterm group had significantly lower placental 1,25(OH)2D3 concentration (4.58±2.90 pg/mg vs 5.57±3.50, p=0.037) and higher placental VDR (21.70 (6.06-73.4) vs 16.48 (1.87-74.67), p=0.041). Placental 25(OH)D and senescence markers between the two groups had no significant difference.

Table 3. Correlation of Vitamin D and Senescence Markers in Term and Preterm Groups

	8-OHdG		GLB1		HMGB1	
	R	***P-value***	**R**	***P-value***	**R**	***P-value***
Maternal serum						
25(OH)D	0.223	0.72	0.166	0.283	0.061	0.624
Placenta						
25(OH)D	-0.235	0.055	-0.337	0.005	-0.149	0.228
CYP27B1	0.256	0.048	0.585	<0.001	0.373	0.003
1,25(OH)2D3	-0.297	0.014	-0.374	0.002	-0.289	0.017
Tissue VDR	-0.046	0.711	0.457	<0.001	0.134	0.277

Table 3 shows the correlation of vitamin D, its receptors, and activating enzymes with senescence markers in term and preterm groups. A significant negative correlation was found between placental 25(OH)D and GLB1 (r=-0.337, p=0.005) as well as T/S ratio (r=-0.252, p=0.040); between 1,25(OH)2D3 and 8-OHdG (r=-0.297, p=0.014), GLB1 (r=-0.374, p=0.002), and HMGB1 (r=-0.289, p=0.017). A significant positive correlation was found between CYP27B1 and 8-OHdG (r=-0.256, p=0.048), GLB1 (r=0.585, p=<0.001), and HMGB1 (r=0.373, p=0.003); and between placental VDR and GLB1 (r=0.457, p=<0.001).

DISCUSSION

Both groups showed very low mean serum 25(OH)D levels, classified as vitamin D deficiency according to the Institute of Medicine (<30 ng/mL). This finding is consistent with previous reports indicating that 99% of pregnant women in Jakarta experience vitamin D deficiency.[8]

Although maternal serum vitamin D levels did not differ significantly between groups, placental $1,25(OH)_2D_3$ levels were significantly higher in the term group, while placental VDR levels were higher in the preterm group. These findings suggest an active role of the placenta in vitamin D metabolism as a compensatory response to low maternal vitamin D status. The placenta expresses CYP27B1, which converts 25(OH)D to $1,25(OH)_2D_3$, a metabolite essential for fetal development, immune regulation, and placental homeostasis through genomic and non-genomic mechanisms. [11,12] Vitamin D promotes immune tolerance and modulates inflammation by upregulating factors such as TLR4 and IDO while suppressing pro-inflammatory cytokines, including IL-1, IL-6, and TNF-α.[13-15]

No significant differences were observed in oxidative stress or senescence markers between the preterm and term groups. Physiological placental senescence normally progresses toward term; however, pathological stress may accelerate this process and contribute to preterm labor. Experimental evidence indicates that senescence-related factors, including HMGB1, promote inflammatory cytokine release, matrix remodeling, and premature membrane rupture, thereby influencing the timing of parturition.[7,16,17]

The negative correlations between placental vitamin D (both precursor and active forms) and oxidative stress and senescence markers suggest a protective role of vitamin D against placental stress. This is consistent with previous findings demonstrating a negative association between 8-OHdG and total antioxidant capacity, a known function of vitamin D.[18] Conversely, the positive correlations between CYP27B1 and VDR with oxidative stress and senescence markers may reflect a compensatory upregulation of vitamin D metabolism and signaling in response to cellular stress. Supporting evidence shows altered VDR expression in relation to trophoblast survival and impaired anti-inflammatory responses in $CYP27B1^{-/-}$ and $VDR^{-/-}$ placental models.[19]

Vitamin D regulates pregnancy-related processes at both genomic and non-genomic levels. Through VDR-mediated gene regulation, it supports immune tolerance and cell cycle control, while non-genomic actions enhance antibacterial defenses, autophagy, and suppression of inflammatory cytokines associated with preterm birth.[20,21]

This study has limitations, including the assessment of a single oxidative stress biomarker and the lack of dietary intake evaluation. Nevertheless, the uniformly low vitamin D status across both groups highlights a potentially modifiable antenatal risk factor. Although maternal serum 25(OH)D alone did not differentiate preterm from term labor, placental pathway differences suggest that early assessment and targeted vitamin D interventions warrant further investigation. Prospective studies incorporating multi-marker senescence panels and supplementation trials are needed to clarify the role of vitamin D in reducing spontaneous preterm birth risk.

CONCLUSION

In conclusion, our findings suggest that placental vitamin D metabolism is associated with biomarkers of oxidative stress and placental senescence, pathways that are implicated in spontaneous preterm birth. Although maternal serum vitamin D levels were comparable between the preterm and term groups, term pregnancies showed higher placental levels of the active metabolite $1,25(OH)_2D_3$, while preterm pregnancies exhibited higher placental VDR expression. This pattern supports the idea that the placenta may locally regulate vitamin D signaling in ways that relate to placental function, including immune regulation and maintenance of tissue homeostasis. Consistent with this, the

correlations observed in our data indicate that variation in placental vitamin D pathway markers co-varies with oxidative stress and senescence markers, but does not establish a protective or causal effect.

The lack of significant between-group differences in oxidative stress and senescence markers also aligns with the view that placental aging is, in part, a physiological process that progresses toward term. At the same time, prior evidence suggests that oxidative stress and inflammatory pathways can contribute to pathological, accelerated senescence in some pregnancies, potentially increasing vulnerability to preterm labor. In this context, the altered expression of placental vitamin D pathway components observed here may reflect adaptive or compensatory regulation in response to a low-vitamin D environment or other unmeasured stressors. Overall, these results should be interpreted as hypothesis-generating and motivate larger, prospective studies to test whether placental vitamin D status or signaling predicts preterm birth risk and whether targeted supplementation can modify relevant biological pathways and clinical outcomes.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to RSUPN Dr. Cipto Mangunkusumo (RSCM) and RS Budi Kemuliaan for providing the facilities, clinical support, and administrative assistance that enabled this research to be conducted. We also thank the healthcare teams and staff at both hospitals for their cooperation during participant recruitment and sample collection.

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