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Original Research

Association between Preterm Premature Rupture of Membranes and Vitamin D Levels in Maternal Plasma and Umbilical Cord Blood of Newborns: A Prospective Study

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Abstract

Background: Preterm premature rupture of membranes (PPROM) is a major cause of preterm birth. There are few reports on vitamin D deficiency associated with PPROM. We aimed to investigate the association between PPROM and vitamin D levels in maternal plasma and the umbilical cord blood of newborns. **Methods**: This prospective study included 355 pregnant women who delivered live infants between March 2017 and December 2018 at a medical center. Vitamin D levels were measured in the maternal plasma at the first, second, third trimesters of pregnancy and just before delivery, and in the umbilical cord blood of newborns at birth. In addition, we evaluated the pregnancy and neonatal outcomes according to vitamin D status. **Results**: The rate of PPROM in the vitamin D deficiency group (25(OH)D <20 ng/mL) was higher than in the non-deficiency group (25(OH)D ≥20 ng/mL) (p = 0.003). Vitamin D levels were significantly lower in the PPROM group than non-PPROM group in maternal plasma [at the first (p = 0.020) and second trimesters (p = 0.029), just before delivery (p = 0.015)], and in the cord blood of newborns (p = 0.006). Multiple logistic regression analysis showed that the odds ratio of PPROM by the increase of 1ng/mL of vitamin D levels in the cord blood of newborns was 0.907 (95% confidence interval 0.836–0.983) after adjustment for other confounders (age, gravidity, parity, and body mass index). **Conclusions**: Vitamin D deficiency has a significant association with PPROM. Our study would aid in understanding the mechanism of prevention of PPROM associated with vitamin D deficiency as well as reduction in preterm births.

Keywords: preterm premature rupture of membranes; vitamin D deficiency; cord blood; inflammation; oxidative stress

1. Introduction

Preterm premature rupture of membranes (PPROM) is defined as a spontaneous rupture of the membranes during pregnancy before 37 weeks' gestation and at least 1 h before the onset of contractions [1]. The incidence of PPROM ranges from 3.0% to 10.0% of all deliveries. Approximately 40% of PPROM cases result in preterm delivery, contributing significantly to increased neonatal morbidity and mortality [2–4]. The main causes of PPROM are infection/inflammation, decidual bleeding, uterine overdistention (e.g., polyhydramnios, twins), genetic predispositions, and smoking [5]. The risk factors for PPROM are generally similar to those for preterm birth (PTB), including preterm spontaneous labor with intact membranes [6]. The known risk factors for preterm birth are short cervical length, polyhydramnios, PPROM, assisted reproductive technologies (ART) procedure, prior PTB, pregnancy induced hypertension, placenta previa, placental abruption, fetal growth restriction, urinary tract infections, complex autoimmune diseases with polytherapy, maternal anxiety, obesity, and malnutrition [7–10]. There are a few reports on maternal vitamin D insufficiency or deficiency associated with PTB [11–13]. Woo *et al*. [14] reported a positive association between vitamin D deficiency and PTB. In contrast, a prospective cohort study reported no significant difference in vitamin D levels between the PTB group and full-term delivery group [15], this association remains conflicting. However, there are few reports that have studied the relationship between PPROM and vitamin D deficiency so far. Therefore, we investigated the association between PPROM and vitamin D levels in maternal plasma and umbilical cord blood of newborns with possible circumstances excluding factors affecting PTB.

2. Materials and Methods

2.1 Study Participants

During the period of March 2017–December 2018, 456 pregnant women were prospectively recruited and followed in the first trimester of pregnancy. Among these women, 101 were excluded for one of the following reasons which could have affected pregnancy outcomes: ART pregnancy (n = 11), twin pregnancy (n = 6), history of intra-abdominal surgery (n = 10), history of uterine cervix surgery (n = 5), concurrent serious medical disease that could affect pregnancy outcomes (for example, type 1 diabetes mellitus, uncontrolled thyroid disease, rheumatoid arthritis, renal disease, uncontrolled hypertension and systemic lupus erythematosus) (n = 10), concordant gynecological problem that could affect pregnancy outcome (leiomyoma >5 cm, and ovary cyst >4 cm) (n = 7), unknown pregnancy outcome due to follow-up loss (n = 52). As a result of the exclusion, 355 pregnant women finally participated in this study (Fig. 1).

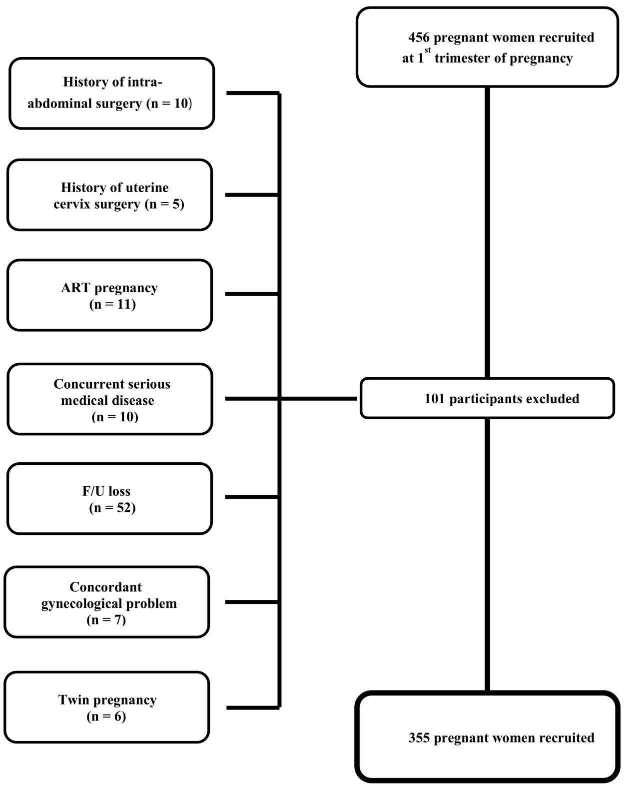

Fig. 1. Flowchart of study participants.

2.2 Data Collection

Participant's demographic characteristics such as age, medical history, operation history, medication, and obstetrical and gynecological history including gravidity and parity were gathered by obstetrical doctors and nurses at the first prenatal consultation. The body mass index (BMI) before pregnancy was collected from the participants' self-reported records and the BMI just before delivery was measured at their admission for delivery. Gestational age was calculated from the last menstrual period and ultrasound measurements. The information regarding the dosage and duration of vitamin D supplementation was collected from the participants' self-reports. Additionally, the birth season was categorized into winter (October to March) and summer (April to September).

2.3 Definitions of Adverse Pregnancy Outcomes

We followed the criterion of PPROM that was defined by a suggestive anamnestic history of vaginal fluid leakage or sonographic evidence of oligohydramnios, followed by documentation of fluid passing through the cervix on sterile speculum examination and a positive nitrazine test (Bristol-Myers Squibb, Princeton, NJ, USA), AmniSure rupture of fetal membrane test (placental alpha microglobulin – 1 or Ferning test) [16]. Gestational diabetes mellitus (GDM) was defined as diabetes diagnosed by glucose tolerance test between 24 and 28 weeks of gestation, excluding overt diabetes mellitus. Pregnancy associated hypertension (HTN) included all hypertensive disorders and their complications during pregnancy. Thyroid disorder included all thyroid diseases diagnosed during pregnancy which were treated with medication. A preterm birth (PTB) was defined as a birth at <37 weeks of gestation.

2.4 Vitamin D in Maternal Plasma and Umbilical Cord Blood

Maternal venous blood (5 mL) was collected in the first trimester (5~9 weeks of gestation), second trimester (24~28 weeks of gestation), third trimester (35~37 weeks of gestation), and just before birth (at their admission for delivery) to measure the levels of vitamin D. Additionally, 5 mL of umbilical venous blood was collected during birth for measuring the levels of vitamin D of newborn baby. Total 25-hydroxyvitamin D (25(OH)D) levels were measured using an electrochemiluminescence binding assay with an automated analyzer (Cobas C 602; Roche Diagnostics, Seoul, Republic of Korea).

Vitamin D deficiency is usually defined as a 25(OH)D level of less than 20 ng/mL (50 nmol/L) [17,18]. The definition of vitamin D deficiency has been a point of debate [19]. The United States Institute of Medicine (USIM) has recently defined levels of serum 25(OH)D above 50 nmol/L (or 20 ng/mL) as adequate in pregnant women [20]. We employed the USIM cutoff for vitamin D deficiency (25(OH)D <20 ng/mL) to analyze its association with adverse pregnancy and neonatal outcomes.

2.5 Statistical Analysis

Continuous variables are presented as medians and interquartile ranges. Categorical variables are presented as numbers and percentages. The patients were divided

Table 1. Characteristics of the Study Participants—PPROM *vs.* non-PPROM groups.

Variables	PPROM (N = 12)	Non-PPROM (N = 343)	*p*
Age, years, median (IQR)	32.5 (27.5–35.5)	33 (30–36)	0.332
BMI, kg/m^2, median (IQR)	21.3 (19.8–28.6)	21.6 (19.7–24.2)	0.533
Gravidity, N (%)			0.134
0	9 (75.0)	182 (53.1)	
≥1	3 (25.0)	161 (46.9)	
Parity, N (%)			0.014
0	11 (91.7)	175 (56.1)	
≥1	1 (8.3)	137 (43.9)	
GDM, N (%)	0 (0)	18 (5.2)	0.999
HTN, N (%)	0 (0)	10 (2.9)	0.999
Thyroid disorder, N (%)	0 (0)	21 (6.1)	0.999
Vitamin D intake, N (%)	5 (41.7)	189 (56.6)	0.306
Birth season, N (%)			0.558
Winter	5 (41.7)	115 (33.5)	
Summer	7 (58.3)	228 (66.5)	
Mode of delivery, N (%)			0.068
Vaginal delivery	2 (16.7)	148 (43.1)	
Cesarean section	10 (6.9)	198 (56.9)	
25(OH)D levels in maternal plasma, ng/mL, median (IQR)			0.034[1] 0.042[2]
1st trimester	8.8 (7.3–12.2)	14.1 (10.3–19.0)	0.02
2nd trimester	12.6 (9.9–24.7)	23.1 (15.9–31.2)	0.029
3rd trimester,	17.8 (13.5–26.9)	24.1 (15.6–33.1)	0.192
Just before birth	14.6 (11.8–20.3)	22.4 (14.1–31.0)	0.015
Cord blood of newborn, ng/mL, median (IQR)	13.4 (9.0–18.1)	20.5 (13.2–28.5)	0.006

PPROM, preterm premature rupture of membranes; BMI, body mass index; GDM, gestational diabetes mellitus; HTN, pregnancy associated hypertension; 25(OH)D: 25-hydroxyvitamin D.
IQR, interquartile range; [1], within-subject effect of repeated measures ANOVA; [2], between-subjects effect of repeated measures ANOVA.

into two groups (Non-PPROM *vs.* PPROM and vitamin D <20 ng/mL *vs.* vitamin D ≥20 ng/mL). Since not all variables were normally distributed, nonparametric methods were used. To compare the two groups, Mann-Whitney U test was used for continuous variables and chi-square or Fisher's exact test were used for categorical variables. Vitamin D levels were measured repeatedly and repeated ANOVA measures were performed. To eliminate the effect of confounding variables, multiple logistic regression analysis was performed. SPSS version 26.0 (IBM, SPSS Statistics, Armonk, NY, USA) was used for statistical analysis. *p*-values were based on a two-sided significance level of 0.05.

2.6 Ethical Statements

This prospective study was conducted after obtaining ethical approval from the Institutional Review Board (IRB) of Seoul Medical Center (2017-03-015). All participants provided written informed consent after receiving a complete explanation of this study.

3. Results

3.1 Basic Characteristics of PPROM vs. Non-PPROM

A total of 355 pregnant women participated in this study from the 1st trimester of pregnancy to delivery, and 12 (3.38%) of them suffered PPROM. Table 1 shows the difference in general characteristics between the PPROM and non-PPROM groups. There was no difference in age, prepregnant BMI, and gravidity between the two groups, however, the incidence of PPROM was significantly higher in the nulliparous group. There was no difference between the two groups in adverse pregnancy outcomes such as GDM, HTN, and thyroid disorders, in birth season and in mode of delivery (Table 1).

3.2 Vitamin D Levels of PPROM vs. non-PPROM

Vitamin D levels in the maternal plasma and umbilical cord blood of newborns were significantly different between the PPROM and non-PPROM groups in the first trimester (*p* = 0.02), second trimester (*p* = 0.029), before birth (*p* = 0.015), and in the umbilical cord blood at birth (*p* = 0.006) (Fig. 2).

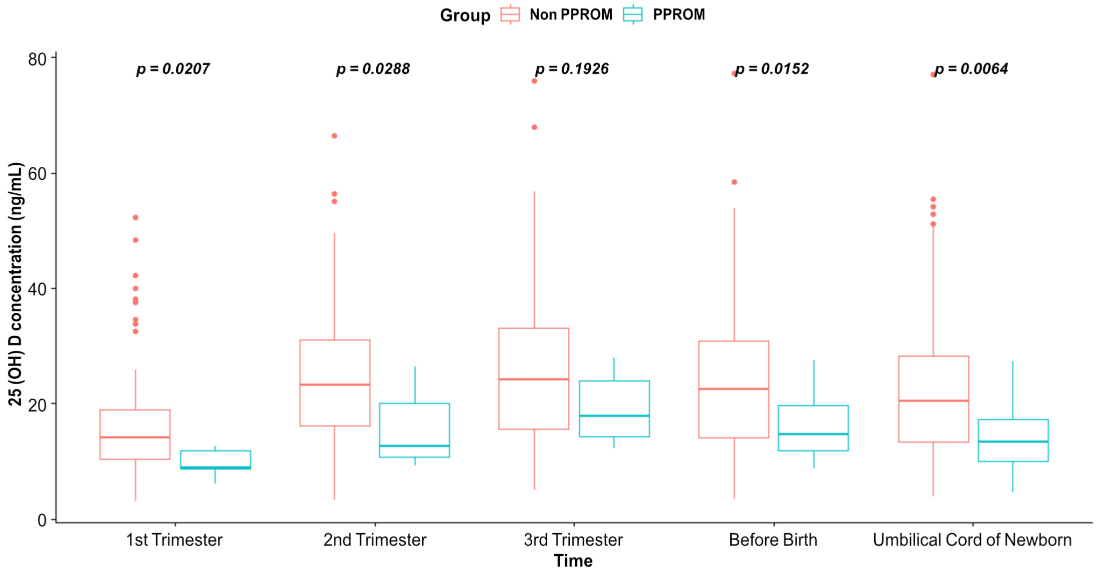

Fig. 2. Comparison and boxplot of vitamin D levels in the maternal plasma and in the umbilical cord blood of newborns.

Table 2. Neonatal outcomes of PPROM *vs.* non-PPROM.

Variables	PPROM	Non-PPROM	p
	(N = 12)	(N = 343)	
Sex, N (%)			0.134
Male	9 (75.0)	182 (53.1)	
Female	3 (25.0)	161 (46.9)	
Birth weight, g, median (IQR)	2925 (2550–3315)	3190 (2920–3500)	0.036
Preterm birth			<0.001
No	4 (33.3)	302 (88.0)	
Yes	8 (66.7)	41 (12.0)	
Apgar score at 1 minute	8 (8–8.75)	8 (8–8)	0.661
Apgar score at 5 minutes	9 (9–9.75)	9 (9–9)	0.962
Neonatal jaundice			0.140
No	7 (58.3)	273 (79.6)	
Yes	5 (41.7)	70 (20.4)	

PPROM, preterm premature rupture of membranes; IQR, interquartile range.

3.3 Neonatal Outcomes of PPROM vs. Non-PPROM

The weight at birth ($p < 0.036$) and the rate of preterm birth ($p < 0.001$) were statistically different between the two groups. There were no statistical differences in the other variables (Table 2).

3.4 Characteristics and Pregnancy Outcomes Compared to Vitamin D Status

Outcomes of pregnancies and newborns were compared by creating two groups based on the vitamin D status of cord blood; the vitamin D deficiency group (<20 ng/mL) and the non-deficiency group (≥20 ng/mL). 176 out of 355 newborns (49.57%) were vitamin D deficient, demonstrating a high deficiency prevalence (Table 3). There was no difference in age between the two groups, however, prepregnant and just before birth BMI were significantly higher in the vitamin D deficiency than in the non-deficiency group. Vitamin D deficiency rate was higher in the multigravida than the primigravida group and in the multiparous than the nulliparous group. The incidence rates of GDM, HTN, and thyroid disorders were not significantly different, however, the incidence of PPROM was found to be significantly higher in the vitamin D deficiency group (p = 0.003).

3.5 Association Between Vitamin D levels and PPROM

Multiple logistic regression analysis was performed to eliminate the confounding effects. After adjusting for other confounders (age, gravidity, parity, and BMI), a higher vitamin D level showed a lower rate of PPROM ($p < 0.018$) (Table 4).

Table 3. Maternal characteristics and pregnancy outcomes according to vitamin D status of cord blood of newborns (total number of participants = 355).

Variables	25(OH)D <20 ng/mL (N = 176)	25(OH)D ≥20 ng/mL (N = 179)	p
Age, years, median (IQR)	33 (30–36)	32 (30–36)	0.509
BMI, kg/m^2, median (IQR)			
Prepregnant	22.5 (20.3–25)	20.9 (19.4–23.4)	<0.001
Just before birth	27.1 (24.7–29.7)	25.4 (23.8–28.2)	<0.001
Gravidity, N (%)			0.002
0	80 (45.5)	111 (62.0)	
≥1	96 (54.5)	68 (38.0)	
Parity, N (%)			0.014
0	60 (34.1)	84 (64.9)	
≥1	116 (65.9)	95 (53.1)	
PPROM, N (%)	11 (6.3)	1 (0.6)	0.003
GDM, N (%)	9 (5.1)	9 (5.0)	0.971
HTN, N (%)	4 (2.3)	6 (3.4)	0.539
Thyroid disorder, N (%)	8 (4.5)	13 (7.3)	0.278
Vitamin D intake, N (%)	68 (40.0)	126 (71.6)	<0.001
Birth season, N (%)			0.433
Winter	56 (31.8)	64 (35.8)	
Summer	120 (68.2)	115 (64.2)	
Mode of delivery, N (%)			0.113
Vaginal delivery	67 (38.1)	83 (46.4)	
Cesarean section	109 (61.9)	96 (53.6)	

PPROM, preterm premature rupture of membranes; GDM, gestational diabetes mellitus; HTN, pregnancy associated hypertension.

Table 4. Multiple logistic regression analysis for PPROM.

Variables	Odds ratio	Confidence interval	p
Age (1 year increase)	0.961	0.855–1.081	0.508
Gravidity (≥1 *vs.* 0)	0.305	0.074–1.258	0.999
Parity (≥1 *vs.* 0)	1.783	0.453–7.020	0.408
BMI (1 kg/m^2 increase)	1.040	0.910–1.189	0.563
25(OH)D levels in the cord blood of the newborn (1 ng/mL increase)	0.907	0.836–0.983	0.018

PPROM, preterm premature rupture of membranes; BMI, body mass index.

3.6 Subanalysis

The results were analyzed only for primigravidas to prevent the previous histories of preterm labor or PTB from affecting the PPROM rate. Among 191 primigravidas, the rate of vitamin D deficiency was 70%. There was no difference in age, BMI, rate of GDM, HTN or thyroid disorders between the vitamin D deficiency and non-deficiency groups, however PPROM occurred only in the vitamin D deficiency group (Table 5).

4. Discussion

Our study was conducted prospectively, and all participants with risk factors that could affect PPROM were excluded. All participants were recruited in the first trimester of pregnancy, and blood was collected for vitamin D level analysis at predetermined periods (the first, second, third trimester of pregnancy and just before delivery). Additionally, vitamin D levels were measured from the blood taken from the umbilical cord of the newborn. Our study showed that the occurrence of PPROM in cases with vitamin D deficiency (<20 ng/mL) in the umbilical cord blood was remarkably higher than that in cases with a normal level of vitamin D. Vitamin D levels in the maternal plasma and umbilical cord blood at birth in the PPROM group were significantly lower than those in the non-PPROM group. The odds ratio of PPROM was 0.907 with an increase of 1 ng/mL of vitamin D level of cord blood. In addition, since the history of preterm labor or PTB can be a risk factor for PPROM, in the subanalysis, only primigravidas were analyzed for the incidence of PPROM. We found that the incidence of PPROM in primigravidas was higher in the vitamin D deficiency group than the normal group. Even when the known

Table 5. Sub-analysis of rate of PPROM on primigravida (total number of participants = 191).

Variables	25(OH)D <20 ng/mL (N = 80)	25(OH)D ≥20 ng/mL (N = 111)	p
Age, years, median (IQR)	31.5 (28–35.7)	31 (30–35)	0.225
BMI, kg/m^2, median (IQR)			
Prepregnant	22.4 (19.8–24.9)	20.8 (19.6–23.5)	0.029
Just before delivery	26.9 (24.4–29.6)	25.5 (24.1–28.6)	0.097
PPROM, N (%)	9 (11.3)	0 (0)	<0.001
GDM, N (%)	4 (5.0)	6 (5.4)	0.999
HTN, N (%)	2 (2.5)	5 (4.5)	0.701
Thyroid disorder, N (%)	5 (6.3)	8 (7.2)	0.796
Vitamin D intake, N (%)	36 (45.6)	88 (80.0)	<0.001
Birth season, N (%)			0.609
Winter	31 (38.8)	39 (35.1)	
Summer	49 (61.3)	72 (64.9)	
Mode of delivery, N (%)			0.415
Vaginal delivery	25 (31.3)	41 (36.9)	
Cesarean section	55 (68.8)	70 (63.1)	

PPROM, preterm premature rupture of membranes; BMI, body mass index; IQR, interquartile range; GDM, gestational diabetes mellitus; HTN, pregnancy associated hypertension.

risk factors, the history of preterm labor and preterm delivery are excluded, PPROM is frequent in the vitamin D deficiency group. Therefore, vitamin D deficiency can be considered another risk factor for PPROM.

The rate of PPROM in this study was 3.38% (12/355), with the rate in the vitamin D deficiency group being 6.3% (11/176) and 0.6% (1/179) in the non-deficiency group. Of these, 66.7% (8/12) led to PTB. In the United States, the PTB rate is approximately 10% [21]. PPROM is responsible for 30%–40% of PTBs [22]. Similar to in the United States, PTB rate is approximately 7% in South Korea [23]. Therefore, reducing vitamin D deficiency during pregnancy might lead to a reduction of PTB due to PPROM.

There are several hypotheses for how vitamin D deficiency can affect PPROM. First, vitamin D is involved in several processes leading to an inflammatory response. The fetal membrane not only acts as a barrier to the invasion of microorganisms [24–26], but also provides immune protection [27–29]. Impairment of these functions of the fetal membrane can lead to microbial invasion in the genital tract as well as triggering of inflammatory processes, resulting in PPROM through collagenolysis [24,30–32]. Some studies have reported that inflammation with bacterial infection is the main cause of PPROM [33], and recent studies have reported that sterile inflammation of the fetal membrane without microbial infection is the predisposing cause of PPROM [34]. Liu *et al.* [35] reported that vitamin D regulates both acquired and innate immune responses at the fetal-maternal interface. Vitamin D could act as an intracrine regulator of cyclic adenosine monophosphate in trophoblasts and regulate innate immune response in the placenta [36,37]. In the placenta, 1,25-dihydroxyvitamin D induces the production of cathelicidin, an antimicrobial peptide, and consequently is associated with a reduction of the risk of bacterial vaginosis. Dunlop *et al.* [38] reported that vitamin D deficiency during pregnancy is more likely to cause bacterial vaginosis. Moreover, sufficient vitamin D levels during pregnancy could reduce the risk of bacterial vaginosis, thereby reducing the incidence of PTB [39,40]. Therefore, maternal vitamin D could function as an immune regulator in pregnancy and have anti-inflammatory effects which reduce the risk of PPROM by decreasing bacterial colonization of the genital tract.

Second, vitamin D is a potent antioxidant. One of the pathophysiologies of PPROM is oxidative stress (OS) mediated by reactive oxygen species (ROS). Collagen is the primary target of ROS. In healthy pregnancies, the ROS and antioxidants are balanced [41–45]. However, this balance is disturbed in case of increase in ROS (high O_2 demand, *e.g.*, high altitude pregnancy, twin pregnancy), antioxidant deficiency, or microvascular disease [46]. Insufficient vitamin D levels are reportedly associated with increased OS or reduced antioxidant capacity [47,48]. Vitamin D promotes the expression of nuclear respiratory factor 2 (Nrf2) which inhibits the action of ROS, and the expression of an antioxidative enzyme that eliminates free radicals at the genetic level [49].

Overall, PPROM is associated with an inflammatory response by infection and directly with OS-inducing chorioamniotic collagenolysis. Vitamin D can regulate the immune response in the placenta and decrease bacterial colonies in the genital tract. Furthermore, vitamin D functions as an antioxidant that upregulates antioxidative enzymes, triggering the expression of Nrf2 that suppresses ROS via genetic mechanism.

We collected pre-pregnancy BMI and measured BMI just before delivery, and Table 3 showed that prepregnant BMI and just before birth BMI were statistically significantly higher in the vitamin D deficiency group than in the normal group. As mentioned in several studies, it was reported that the higher the BMI, the lower the vitamin D blood concentration [50], and there is also a research result that the bioavailability of vitamin D is lowered in the obesity group [51].

Our study has several advantages. This study was prospectively conducted. Therefore, it was possible to exclude factors that could affect PPROM. Additionally, to exclude previous PTB and preterm labor history, a second sub-analysis was performed with only primigravidas. Our sample size of 355 pregnant women was relatively larger than that of previous studies. Moreover, this study investigated the association between PPROM and vitamin D deficiency in the umbilical venous cord blood of newborns. To the best of our knowledge, previous studies on this association are scarce.

However, our study has some limitations. The sample size was too small to allow the generalizability of our research outcomes. Additionally, we could not accurately analyze the correlations between the vitamin D level in the maternal plasma, umbilical cord blood, and vitamin D intake (through vitamin D therapy or diet during pregnancy). In the future, further study on PPROM and vitamin D levels, considering fetal infection and vitamin D intake, is required to study the detailed mechanism of PPROM associated with vitamin D deficiency.

5. Conclusions

PPROM accounts for a considerable portion of PTB, which increases fetal morbidity and mortality. In this study, we found that vitamin D deficiency had a significant association with PPROM. We expect that the incidence of PPROM could be considerably reduced if vitamin D intake is recommended before and during pregnancy. For this to be achieved, further large-scale research needs to be conducted.

Author Contributions

HJL—Designed the research study, acquired patient data, and drafted the manuscript; JYH—Conception, design, statistical analysis, created figures and tables, provided guidance, responsible for the accuracy and integrity of the work presented here; JHH—Statistical analysis and data extraction; HYK—Interpretation of data and statistical analysis; HZC—Statistical analysis and data extraction, created figures and tables. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript.

Ethics Approval and Consent to Participate

This prospective study was conducted after obtaining ethics approval from the institutional review board of Seoul Medical Center (2017-03-015). All participants provided written informed consent after receiving a complete explanation of this study.

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Conflict of Interest

The authors declare no conflict of interest.

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