

## Research Article

## Maternal Vitamin D and Its Role in Amniotic Membrane Thickness in Premature Rupture of Membranes

Hafis Novyan, Rajuddin Rajuddin, Niken Asri Utami

*Department obstetrics and Gynecology  
Faculty of Medicine Universitas Syah Kuala  
Dr. Zainoel Abidin General Hospital  
Banda Aceh*

### Abstract

**Objective:** To examine the correlation between maternal serum vitamin D levels and amniotic membrane thickness in cases of premature rupture of membranes (PROM).

**Methods:** This analytical cross-sectional study was conducted from July to November 2022 at Dr. Zainoel Abidin General Hospital, the Universitas Syiah Kuala Research Laboratory, and Prodia Banda Aceh Laboratory. A total of 30 women with term PROM who met the inclusion and exclusion criteria were enrolled. Serum vitamin D levels were measured using Electrochemiluminescence Immunoassay (ECLIA), while amniotic membrane thickness was assessed histologically using hematoxylin Eosin (HE) staining. Spearman's correlation test was performed with a 95% confidence level.

**Results:** Thirty mothers with PROM were included, with a mean age of  $31.37 \pm 6.8$  years. Vaginal delivery was the predominant mode of delivery (53.3%). The mean serum vitamin D level was 20.25 ng/mL (range: 8.5–42.5 ng/mL), and the mean amniotic membrane thickness was 82.51  $\mu\text{m}$  (range: 28.69–227.8  $\mu\text{m}$ ). No significant correlation was observed between serum vitamin D levels and amniotic membrane thickness ( $r = -0.029$ ;  $p = 0.878$ ).

**Conclusion:** There was no significant correlation between maternal serum vitamin D levels and amniotic membrane thickness in cases of PROM.

**Keywords:** amnion, pregnancy, premature rupture of membranes, thickness of the membranes, vitamin D.

**Correspondence author.** Rajuddin. Department of Obstetrics and Gynecology.  
Faculty of Medicine Universitas Syah Kuala. Dr. Zainoel Abidin General Hospital. Banda Aceh.  
Email; rajuddin@usk.ac.id

### INTRODUCTION

Vitamin D is a micronutrient that exists in the human body that plays an important role in various metabolic processes, including the regulation of inflammation. During pregnancy, the requirement for vitamin D increases.<sup>1</sup> Vitamin D insufficiency among pregnant women is relatively high, ranging from 20% to 90%, as reported in several studies. This high prevalence is associated with inadequate dietary intake and limited exposure to ultraviolet light. The Endocrine Society defines vitamin D deficiency as a serum level  $<20$  ng/mL ( $<50$  nmol/L).<sup>1</sup> Vitamin D deficiency during pregnancy has been associated

with adverse maternal and fetal outcomes, including gestational diabetes, preeclampsia, preterm birth, asthma, and impaired psychomotor and cognitive development in children.<sup>2</sup>

Individuals with low vitamin D intake have an increased risk of chronic inflammatory diseases, such as allergies, infections, autoimmune disorders, cardiovascular disease, and cancer.<sup>3</sup> Vitamin D is associated with Matrix Metalloproteinase (MMP) activity in nasal and lung polyps. Increased MMP production during inflammatory processes contributes to collagen degradation. Collagen is a major structural component of the amniotic membrane.<sup>4</sup>

The amniotic sac, a component of the fetal membranes, is continuously hydrated by amniotic

fluid. It is highly elastic and resistant to pressure, preventing rupture during normal pregnancy. The amniotic membrane consists of a thick basement membrane composed of collagenous tissue interspersed with mesenchymal cells that support a monolayer of epithelial cells. Dense connective tissue layers and fibroblasts underlying the basement membrane form the primary fibrous framework of the amniotic membrane. Parallel bundles of collagen fibrils, predominantly interstitial collagen, maintain the mechanical integrity of the membrane.<sup>5</sup>

Collagen degradation is mediated by matrix metalloproteinases (MMPs), enzymes that hydrolyze components of the extracellular matrix. Gelatinases such as MMP-2 and MMP-9 degrade the triple-helical structure of collagen types I and II, which are initially cleaved by MMP-1 and MMP-8. Type IV collagen, fibronectin, and proteoglycans are also substrates for gelatinases. In fetal membranes, MMP-1 and MMP-9 are primarily produced by the amniotic and chorionic epithelium. Excessive collagen degradation in the amniotic membrane may ultimately lead to Premature Rupture of Membranes (PROM).<sup>6</sup>

Previous studies have shown that patients with PROM have significantly lower levels of type III collagen compared to those without PROM ( $350 \pm 70 \mu\text{g}/\text{mg}$  vs.  $587 \pm 84 \mu\text{g}/\text{mg}$ ,  $p < 0.01$ ).<sup>7</sup> Significantly lower serum vitamin D levels in women with PROM ( $13.75 \pm 7.21 \text{ ng}/\text{mL}$ ) compared to those with preterm labor ( $18.83 \pm 6.07 \text{ ng}/\text{mL}$ ;  $p = 0.000$ ) and term pregnancies ( $17.38 \pm 6.65 \text{ ng}/\text{mL}$ ;  $p = 0.009$ ).<sup>8</sup> An experimental animal study demonstrated that vitamin D can inhibit placental inflammation through activation of the Vitamin D Receptor (VDR) and modulation of the NF- $\kappa$ B pathway.<sup>9</sup> Similarly, found that women with PROM had lower vitamin D levels.<sup>10</sup>

The association between vitamin D deficiency, inflammation, and increased MMP production may contribute to collagen degradation in the amniotic membrane, potentially leading to PROM. Pregnant women with PROM are reported to have lower vitamin D levels. Therefore, this study aims to examine the relationship between maternal serum vitamin D levels and amniotic membrane thickness in PROM cases.

## METHODS

This analytic study with a cross-sectional design was conducted at dr. Zainoel Abidin General Hospital, Prodia Laboratory, and the

Research Laboratory of Universitas Syiah Kuala. Ethical approval was obtained from the Health Research Ethics Committee of dr. Zainoel Abidin General Hospital Banda Aceh (No. 1171012P). The study was carried out from July 1 to November 30, 2022.

The study population consisted of patients with Premature Rupture of Membranes (PROM) who presented to the emergency department of dr. Zainoel Abidin General Hospital Banda Aceh. The sample size was calculated using the appropriate formula, resulting in a total of 30 participants who met the inclusion and exclusion criteria.

The inclusion criteria were: adults aged  $>18$  years, patients diagnosed with PROM, and those with term gestational age. The exclusion criteria were: patients with signs and symptoms of chorioamnionitis; PROM with polyhydramnios; PROM caused by invasive procedures (amniocentesis or cordocentesis); and patients who refused to participate in the study.

Eligible subjects were informed about the purpose and benefits of the study and were asked to provide written informed consent prior to participation. Peripheral venous blood samples were collected and sent to Prodia Laboratory for measurement of serum 25-hydroxyvitamin D [25(OH)D] levels using the Electrochemiluminescence Immunoassay (ECLIA) method.

After delivery, samples of the amniotic membrane attached to the placental margin were collected and examined at the Research Laboratory of Universitas Syiah Kuala. The tissue samples were processed and stained using Hematoxylin–Eosin staining. The thickness of the amniotic membrane was measured microscopically.

Demographic and clinical characteristics collected included maternal age, Body Mass Index (BMI), gestational age, education level, parity, mode of delivery, and history of vitamin D supplementation.

Descriptive data are presented in tables and graphs using univariate analysis, expressed as frequencies and percentages for each variable. The Pearson correlation test was used to analyze the relationship between maternal serum vitamin D levels and amniotic membrane thickness in cases of PROM if the data were normally distributed. If the data were not normally distributed ( $p < 0.05$ ), the non-parametric Spearman rank correlation test was applied. A 95% confidence level was used,

and the strength of the correlation was assessed using the correlation coefficient (r value).

**Table 1.** Characteristics of Research Subjects

Characteristic	Mean / frequency	P-value
Age (year), mean $\pm$ SD	31.37 $\pm$ 6.08	0.501 <sup>a</sup>
IMT (Kg/m <sup>2</sup> ), mean $\pm$ SD	26.17 $\pm$ 4.77	0.735 <sup>a</sup>
<b>Gestational age (weeks), n (%)</b>		0.134 <sup>b</sup>
37 – 38	11 (36.7)	
38 – 39	10 (33.3)	
39 – 40	7 (23.3)	
40 – 41	2 (6.7)	
<b>Vitamin D consumption history, n (%)</b>		0.842 <sup>c</sup>
Yes	5 (16.7)	
No	25 (83.3)	

Description: VD: Vaginal Delivery; CS: cesarean section.

aLevel of significance of Pearson test

bLevel of significance of ANOVA

cLevel of significance of T-test

Table 1 above shows the study subjects' average age was 31.37 years with a nutritional status based on a IMT rating of 26.17 kg/m<sup>2</sup>. Based on their educational status, the research subjects were dominated by the high school education level with a percentage of 50%. Based on the status of the pregnancy, it was dominated by multigravidas with a distribution of 53.3% by PV method of delivery and 46.7% by SC. A total

of 5 research subjects had a history of vitamin D supplementation with a percentage of 16.7%. The baby outcome were born from the study subjects APGAR scores at 1 and 5 minutes respectively were at good score and indicated that the newborn was not asphyxiated. The born neonates showed normal birth weight with an average of 2,876.67 (SD $\pm$ 350,94) grams.

**Table 2.** Research Variable Correlation Analysis

	Median	Min – Max	R	P-value*
Vitamin D levels	20.25	8.5 – 42.5	-0.029	0.878
Amniotic membranes thickness	82.51	28.69 – 227.8		

Information: \*Spearman's Rank correlation test

Table 3 above presents data on the correlation test results between the levels of the serum vitamin D and the thickness of the amniotic membranes. Based on Shapiro-Wilk test, both Vitamin D and Amniotic membrane thickness were not normally distributed. The subjects' mean serum vitamin D level in this study was 20.25 ng/ml which was included in the category of serum vitamin D insufficiency. The thickness of the amniotic membranes at the edge of placenta showed a mean value of 82.51  $\mu$ m. Statistically, based on Spearman's Rank correlation test, it was discovered that between maternal serum vitamin D levels and maternal amniotic membrane thickness with premature rupture of membranes ( $p > 0.05$ ) has no significant correlation.

## DISCUSSION

### Characteristics of the Study Subjects

The mean age of the study participants was 31.37 years. This finding is consistent with previous research by Vanesha, which reported that 95% of patients with premature rupture of membranes (PROM) were women aged 20–35 years.<sup>11</sup> Similarly, a study found that 81.66% of PROM cases occurred in women of reproductive age.<sup>12</sup> In the present study, 36.7% of participants were primigravidas. This finding is comparable to the results reported by Amulya, who observed that 71.16% of PROM cases occurred in primigravid women.<sup>12</sup> Fiftyfive percents of patients with PROM were primigravidas.<sup>11</sup> These findings suggest that

primigravidity may be a common characteristic among women experiencing PROM.

### Amniotic Membrane Thickness

Previous studies have demonstrated significant differences in amniotic membrane thickness and collagen content between cases with PROM and those without PROM. This finding indicates that collagen, a major structural component of the amniotic membrane, plays a crucial role in maintaining membrane integrity, which may be compromised due to increased matrix metalloproteinase (MMP) activity induced by inflammation.<sup>7</sup> Higher MMP levels in cases of preterm premature rupture of membranes (PPROM) compared to PROM.<sup>13</sup>

These findings are consistent with those reported by Bhunia, who demonstrated that ruptured areas of the amniotic membrane were significantly thinner than non-ruptured areas (0.77 mm vs. 0.40 mm;  $p = 0.0001$ ). Sampling location has been suggested to influence measured membrane thickness.<sup>14</sup> Thinning of the amniotic membranes significantly increases the risk of premature rupture. A significantly higher risk of membrane rupture in thinner regions, with a chorion thickness of 147.2  $\mu\text{m}$  in the PROM group compared to 253.7  $\mu\text{m}$  in the normal pregnancy group ( $p < 0.0001$ ).<sup>15</sup>

### Obstetric Causes and Outcomes of Vitamin D Deficiency

Insufficient sunlight exposure, darker skin pigmentation, and inadequate dietary intake are among the main factors contributing to vitamin D deficiency. In Turkey, the high prevalence of vitamin D deficiency has been attributed to nutritional factors, lifestyle, and environmental conditions. Adverse obstetric outcomes associated with low serum 25-hydroxyvitamin D levels include preterm birth, low birth weight, increased rates of cesarean section, gestational diabetes, and hypertensive disorders of pregnancy.<sup>2</sup>

Several studies have reported no significant association between vitamin D deficiency and placental inflammation or amniotic membrane thickness. However, an association has been observed when maternal vitamin D levels are markedly low ( $<P5$ ; OR = 1.61;  $p = 0.012$ ). This association remained significant after adjustment for maternal age (OR = 1.57;  $p = 0.017$ ), gestational

age at delivery, neonatal birth weight, neonatal sex, and timing of sample collection (OR = 1.53;  $p = 0.027$ ). These findings suggest that low vitamin D levels, particularly during the first trimester, may be associated with placental inflammation, although vitamin D deficiency alone may not be the sole determining factor.<sup>3,14</sup>

Vitamin D deficiency has been shown to increase inflammatory activity and subsequently elevate MMP activity in tissues. Increased MMP activity is associated with collagen degradation, which may reduce the tensile strength of the amniotic membrane.<sup>4-6</sup> In the present study, the mean amniotic membrane thickness was 82.51  $\mu\text{m}$ . Based on Spearman's rank correlation analysis, no significant correlation was found between maternal serum vitamin D levels and amniotic membrane thickness in PROM cases ( $p > 0.05$ ). This finding suggests that vitamin D may influence membrane tensile strength rather than membrane thickness.

### Vitamin D Supplementation

Vitamin D supplementation is intended to increase circulating serum vitamin D levels. However, the optimal supplementation dose required to achieve adequate serum levels remains uncertain, as it is influenced by several factors, including body fat percentage, calcium intake, and hepatic hydroxylation of vitamin D.<sup>16</sup> In this study, five participants (16.6%) reported a history of vitamin D supplementation; however, only one of these participants had normal serum vitamin D levels. Among these five participants, the thickness of the amniotic membranes was within the normal range.

### Study Limitations

This study has several limitations. First, the sample size was relatively small, consisting of only 30 participants. Second, potential confounding factors such as body mass index, gestational age, socioeconomic status, gastrointestinal absorption, and other maternal factors may have influenced amniotic membrane thickness. Third, membrane samples were collected from a single anatomical location; previous studies have shown that amniotic membranes near the placental margin are thicker than those in the pericervical area. Finally, other biochemical factors were not assessed, despite the possibility that vitamin D may influence MMP activity and consequently

affect the tensile strength of amniotic membrane collagen.

### CONCLUSION

Maternal serum vitamin D levels and the thickness of the amniotic membranes in the cases of PROM has no relationship.

### ACKNOWLEDGEMENT

Researchers are grateful for the participation and support given by all samples, staff of the Department of Obstetrics and Gynecology, Faculty of Medicine, Syiah Kuala University / RSUD dr. Zainoel Abidin Banda Aceh in this study.

### REFERENCES

- Dhamayanti M, Noviandhari A, Supriadi S, Judistiani R, Setiabudiawan B. Association of maternal vitamin D deficiency and infants' neurodevelopmental status: A cohort study on vitamin D and its impact during pregnancy and childhood in Indonesia. *J Paediatr Child Health*. 2020;56(1):16-21. doi: 10.1111/jpc.14481. Epub 2019 May 6.
- Fernando M, Ellery S, Marquina C, Lim S, Naderpoor N, Mousa A. Vitamin D-Binding Protein in Pregnancy and Reproductive Health. *Nutrients*. 2020;12(5):1489. doi: 10.3390/nu12051489
- Zhang Q, Chen H, Wang Y, Zhang C, Tang Z, Li H. Severe vitamin D deficiency in the first trimester is associated with placental inflammation in high-risk singleton pregnancy. *Clin Nutr*. 2019;38(4):1921-6. doi: 10.1016/j.clnu.2018.06.978. Epub 2018 Jul 9.
- Wang L, Tai C, Chien C, Chiang F, Chen J. Vitamin D decreases the secretion of matrix metalloproteinase-2 and matrix metalloproteinase-9 in fibroblasts derived from Taiwanese patients with chronic rhinosinusitis with nasal polyposis. *Kaohsiung J Med Sci*. 2015;31(5):235-40. doi: 10.1016/j.kjms.2015.02.001. Epub 2015 Mar 6
- Connon C, Nakamura T, Hopkinson A, Quantock A, Yagi N, Douth J. The Biomechanics of Amnion Rupture: An X-Ray Diffraction Study. *PLoS ONE*. 2007;2(11):e1147.
- Burzle W. Mechanical characterization and modeling of human fetal membrane tissue. ETH Zurich. Doctoral Thesis. 2014. doi.org/10.3929/ethz-a-010147119.
- Marpaung J. Association between the thickness of the collagen in the amniotic membrane with the incidence of premature rupture of membranes. *Int J Reprod Contracept Obstet Gynecol*. 2016;5(2):296-9. doi: http://dx.doi.org/10.18203/2320-1770.ijrcog2016035.
- Mehndiratta MM, Gupta BK, Kar R, Garg S, Anthonio A, Kumari R. 25-Hydroxyvitamin D Insufficiency in Pregnant Indian Women and the Development of Preterm Prelabour Rupture of Membranes. *J Obstet Gynecol India*. 2021;71(6):649-50. doi: 10.1007/s13224-021-01526-6. Epub 2021 Aug 6.
- Liu N, Kaplan A, Lagishetty V, Ouyang Y, Ouyang Y, Simmons C. Vitamin D and the Regulation of Placental Inflammation. *J Immunol*. 2011;186(10):5968-74. doi: 10.4049/jimmunol.1003332. Epub 2011 Apr 11.
- Kassai MS, Cafeo FR, Affonso-Kaufman FA, Suano-Souza FI, Sarni ROS. Vitamin D plasma concentrations in pregnant women and their preterm newborns. *BMC Pregnancy and Childbirth*. 2018;18(1):412. doi: 10.1186/s12884-018-2045-1.
- Vanessa V, Wantania J, Lengkong RA. Vaginal Microorganism Pattern in Premature Rupture of Membrane: Pola Mikroorganisme Vagina pada Ketuban Pecah Dini. *Indones J Obstet Gynecol*. 2019;4(2):198-203. doi: https://doi.org/10.32771/inajog.v9i4.1350.
- MN A, MS A. Maternal outcome in term premature rupture of membranes. *Int J Reprod Contracept Obstet Gynecol*. 2019;8(2):576-9. doi: http://dx.doi.org/10.18203/2320-1770.ijrcog20190287.
- Rajuddin R, Putri T, Munawar M. C-Reactive Protein and Matrix Metalloproteinase-7 in Preterm Premature Rupture of Membranes (PPROM) and Premature Rupture of Membranes (PROM). *Indones J Obstet Gynecology*. 2022;10(3):139-44. doi: https://doi.org/10.32771/inajog.v10i3.1574
- Bhunias S, O'Brien S, Ling Y, Huang Z, Wu P, Yang Y. New approaches suggest term and preterm human fetal membranes may have distinct biomechanical properties. *Scientific Reports*. 2022;12(1):5109.
- Fortner K, Grotegut C, Ransom C, Bentley R, Feng L, Lan L. Bacteria Localization and Chorion Thinning among Preterm Premature Rupture of Membranes. *PLoS ONE*. 2014;9(1):e83338.
- Ramasamy I. Vitamin D Metabolism and Guidelines for Vitamin D Supplementation. *The Clinical Biochemist Reviews*. 2020;41(3):103-26. doi: 10.33176/AACB-20-00006.