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COMPARISON OF VITAMIN D LEVELS BETWEEN MOTHERS AND INFANTS WITH AND WITHOUT PROLONGED MEMBRANE RUPTURE

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СРАВНЕНИЕ УРОВНЕЙ ВИТАМИНА D У МАТЕРЕЙ И НОВОРОЖДЕННЫХ С ДЛИТЕЛЬНЫМ РАЗРЫВОМ ПЛОДНЫХ ОБОЛОЧЕК И БЕЗ НЕГО

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Abstract

Background: Premature membrane rupture is a known causes of preterm labor and accounts for approximately one-third of cases. Vitamin D deficiency may play a role in preterm labor as well.

Objective: The present study aimed to compare vitamin D levels in mothers and infants with and without prolonged rupture of membranes (PROM).

Materials & Methods: This cross-sectional study was conducted with 241 babies, with and without a history of membrane rupture, in mothers in the neonatal ward of Ghaem Hospital, Mashhad, from 2019 to 2021 with available sampling methods. After confirmation of prolonged membrane rupture (more than 18 hours before birth) based on history and examination with a speculum, we completed a data collection tool, a checklist including laboratory evaluation and neonatal and maternal characteristics. The conditions of neonates with and without prolonged membrane rupture were compared. Data were analyzed by T-test and Chi-square.

Results: We examined a total of 241 neonates including 148 (61.4%) without prolonged rupture of the membranes in mothers and 93 (38.6%) with PROM. There were statistically significant differences between the two groups regarding: maternal vitamin D level (p = 0.001); neonatal vitamin D level (p = 0.001); and fifth minute Apgar score (p = 0.003). These variables were lower in the group of neonates with PROM.

Conclusion: Vitamin D deficiency in mothers was significantly associated with prolonged membrane rupture. With increasing severity of vitamin deficiency, the probability of PROM increases and, therefore, the likelihood of preterm labor and its complications rises.

Keywords: fetal membranes, prolonged premature rupture of membranes, infant, newborn, vitamin D, mothers, Apgar score

Резюме

Актуальность: Преждевременный разрыв плодных оболочек является одной из известных причин преждевременных родов и составляет примерно одну треть всех случаев. Дефицит витамина D также может играть роль в преждевременных родах.

Цель: настоящее исследование направлено на сравнение уровней витамина D у матерей и младенцев с пролонгированным разрывом плодных оболочек и без него.

Материалы и методы. Настоящее поперечное исследование было проведено на 241 младенце с разрывом плодных оболочек у матерей и без него в неонатальном отделении больницы Гаем, Мешхед, с 2019 по 2021 год с использованием доступного метода выборки. После подтверждения длительного разрыва плодных оболочек (более 18 часов до рождения) на основании анамнеза и осмотра с помощью зеркал мы заполнили инструмент для сбора данных, контрольный список, включающий лабораторную оценку и неонатальные и материнские характеристики. Сравнивали состояние новорожденных с длительным излитием плодных оболочек и без него. Данные анализировали с помощью Т-критерия и Хи-квадрата.

Результаты. Обследован 241 новорожденный, в том числе 148 (61,4%) без длительного разрыва плодных оболочек у матерей и 93 (38,6%) с ПРПО. Между двумя группами обнаружена статистически значимая разница в уровне витамина D у матери (P = 0,001), уровне витамина D у новорожденного (P = 0,001) и оценке по шкале Апгар на пятой минуте (P = 0,003). Это означает, что значения указанных переменных были ниже в группе новорожденных с ПРПО.

Вывод: Дефицит витамина D у матерей в значительной степени связан с длительным разрывом плодных оболочек, а с усилением тяжести дефицита витамина D увеличивается вероятность ПРПО и, следовательно, повышается вероятность преждевременных родов и их осложнений.

Ключевые слова: плодные оболочки, пролонгированный преждевременный разрыв плодных оболочек; младенец, новорожденный; витамин D, матери, оценка по шкале Апгар

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Introduction

With a prevalence of 11% worldwide, preterm labor is one of the causes of death and long-term disability of infants and remains a major concern for public health (1). The amniotic membrane protects the fetus against inflammation, bacteria, and viruses. Prolonged rupture of the membranes means rupture of the amniotic sac more than 18 hours before delivery (2), and it is a risk factor for early neonatal sepsis (3). Its prevalence is reported to be 4-19%(4), and it is a predisposing factor for about one-third of preterm labors (5). Maternal risk factors for premature rupture of membranes include age, parity, education, hypertension, cervical length with a history of miscarriage, history of upper urinary tract infection, sexually transmitted infections, positive vaginal culture, history of PROM, addiction, diabetes, placental abruption, preeclampsia, and cerclage. Labor complications of premature membrane rupture involve cesarean section, oligohydramnios, chorioamnionitis, placental abruption, fetal distress, fever at the time of delivery, placenta previa, infection, prenatal bleeding, sepsis in the mother, need for antibiotic therapy, placental retention, and post-partum endometriosis. Prematurity, respiratory distress syndrome, asphyxia, infection, meningitis, sepsis, pneumonia, perinatal mortality, patent arterial duct, necrotizing enterocolitis, intraventicular hemorrhage, and pulmonary hypoplasia are neonatal complications of PROM (6).

Low levels of maternal vitamin D during pregnancy are a risk factor for many adverse outcomes, including preterm labor (7). The prevalence of vitamin D deficiency in pregnant women in the first and second trimesters is significantly higher than in the third trimester(8). The fetus relies exclusively on the maternal concentration of 25 hydroxyvitamin D (9). Vitamin D levels in pregnancy are associated with many maternal and fetal health outcomes. There have been numerous studies regarding an association between 25-hydroxyvitamin D levels in pregnancy and multiple pregnancy complications including gestational hypertension, preeclampsia, gestational diabetes, time and type of delivery, preterm labor, and fetal complications including skeletal, immune, and respiratory

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system problems (10, 11). Vitamin D deficiency in premature neonates is associated with a number of problems (e.g. cerebral hemorrhage, retinopathy of prematurity, infection, and even death) (12). Thus, correction of maternal vitamin D levels reduces the incidence of prematurity and these important neonatal problems. In the study of Shah et al., 25-hydroxyvitamin D levels were positively associated with fetal intrauterine growth restriction and prolonged rupture of membranes (13). In general, prolonged membrane rupture is a serious complication in pregnancy, and it can increase mortality or perinatal morbidity, especially preterm labor and infection (14). Due to the association between vitamin D deficiency and prematurity, one of the mechanisms of prematurity in vitamin D deficiency may be a membrane rupture. Therefore, in this study, we compared vitamin D levels in infants and mothers with and without a prolonged membrane rupture.

Materials & Methods

This cross-sectional study was conducted in the maternity ward, midwifery ward, and NICU of Ghaem Hospital in Mashhad from 2019 to 2020. Prior to enrollment, verbal consent was obtained from the infant's parents. According to Zhang Q's study: the prevalence of placental inflammation in mothers with vitamin D deficiency is 63%; and in mothers without placental inflammation, it is 37%. Using a formula to compare two ratios related to a qualitative trait from two communities, along with alpha coefficient of 0.01 and beta of 0.2, the sample size in each group was estimated to be 87 mothers (15).

Mothers who gave birth to premature infants with more than 18 hours of ruptured amniotic sac were studied as a case group. Mothers without prolonged membrane rupture with preterm labor were included in the study as a control group. Confirmation of ruptured amniotic sac was based on history and speculum examination. We completed the data collection tool, a checklist including laboratory evaluation, and neonatal and maternal characteristics. Preterm labor is a delivery before 37 weeks of gestation with or without membrane rupture (16). Premature rupture of the amniotic sac means spontaneous rupture of the fetal

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membranes before 37 weeks of gestation and before the onset of uterine contractions (17). Prolonged rupture of the membranes means rupture of the amniotic sac more than 18 hours before delivery (18).

Exclusion criteria included amniotomy, urinary tract infection, vaginal infection, or congenital anomalies. Serum vitamin D was measured from mothers and umbilical cords during delivery, and 1.5 cc of the prepared samples were centrifuged, with serum kept at -20°C and sent for laboratory evaluation. Vitamin D levels were measured using an ELISA method and the model RT2100c reader made in Germany and an ELISA washing device. We considered less than 30 mg/ml of vitamin D as deficient and above 30 mg/ml as sufficient. Vitamin D deficiency cases were divided into three groups: severe deficiency below 10 mg/ml; moderate deficiency 10-20 mg/ml; and mild deficiency 20-30 mg/ml.

Infants in the two groups were compared in terms of neonatal, maternal, and laboratory characteristics. Checklist status was completed based on neonatal information (gestational age, first minute Apgar score, fifth minute Apgar score), maternal information (maternal age, parity), and blood tests (maternal and neonatal vitamin D levels, C-reactive protein, erythrocyte sedimentation rate, white blood cell, nucleated red blood cell). All tests, except for the vitamin D test, were requested by the treating physician, and we recorded them without intervention.

After discharge, they were followed up using the Denver II test at 24 months. The Denver Developmental Screening Test II, to assess the growth and development of children from birth to six years old, is divided into four categories: personal/social; fine motor; gross motor; and language. If the infant had a problem in every category (i.e., fine motor skills, gross motor skills, language and personal/social), it would be considered as developmental delay for them. If the infant had: a problem in only one category, it is considered a mild developmental delay; in two categories, a moderate developmental delay; and in three or more categories, a severe developmental delay []. A favorable outcome was defined as normal neurologic and good general condition at the end of the study. Unfavorable

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outcome was defined as the presence of at least delay in one domain of Denver screening (19).

Statistical analysis

Data were analyzed using *t*-test, chi-square, and SPSS software version 20. First, we described the results using statistical tables and graphs. We then compared the two groups of infants, with or without prolonged rupture of membrane in mothers, using chi-square and *t*-test. Correlation methods were used to evaluate the association between the severity of vitamin D deficiency and PROM. The $p \leq 0.05$ level was considered significant in all cases.

Ethical consideration

This study was approved by the Ethics Committee of the Vice Chancellor for Research of Mashhad University of Medical Sciences (No 991476, IR.MUMS.MEDICAL.REC.1399.623).

Results

In this study, 9 infants were excluded (3 cases of amniotomy, 2 cases of urinary tract infection, 3 cases of vaginal infection, 1 case of congenital anomaly). Finally, we examined 241 neonates including 148 infants without prolonged rupture of membrane and 93 infants with PROM. Based on the results of this study, the mean gestational age was 33.57 ±3.33 weeks, and the mean maternal vitamin D level was 20.66 ±13.03 mg/dL. Other characteristics of the studied infants are given in Table 1.

Table 1: Means of variables of mothers and newborns included in the study

In addition, 36 infants (14.9%) needed resuscitation; 28.6% were born naturally, and 71.4% by cesarean section. In this study, there were statistically significant differences: between maternal vitamin D levels ($p = 0.001$) and neonatal vitamin D levels ($p = 0.001$); in fifth minute Apgar score ($p = 0.003$); and in CRP ($p = 0.032$) (Table 2).

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Table 2: Comparison of variable means of mothers and neonates with or without prolonged rupture of membranes

Calculations were performed based on standard deviation±mean (***t*-test**).

Twenty percent of infants with PROM had an infection (16% sepsis, 4% meningitis), while 5% of neonates without a history of PROM had an infection. In this study, vitamin D deficiency was significantly associated with prolonged rupture of membranes (p = 0.0001), and with increasing severity of vitamin D deficiency, the rate of PROM increased. Mothers with normal vitamin D level had a 29% chance of PROM, which increased to 47% in moderate deficiency and 62% in severe deficiency. There was a moderate association between maternal serum vitamin D and the incidence of PROM (p = 0.0001 , Spearman's rho = 0.286). Short-term neonatal follow-up showed that the serum level of vitamin D in PROM infants who died was 9.76 ± 1.01 and 12.92 ± 7.67 mg/ml in discharged live neonates (p = 0.003). Assessment of neonatal development from PROM mothers by Denver II test showed that 35% of neonates at 24 months of age showed some degree of developmental delay. In neonates from mothers without PROM, 12% showed developmental delay (p = 0.001). Serum vitamin D levels in children with developmental delay were 13 ± 7. In the group with normal development, they were 17 ± 11 mg/ml (p = 0.042).

Discussion

According to the results of our study, there was a significant relationship between vitamin D deficiency in mothers and infants and prolonged rupture of the membranes, with the likelihood of PROM rising as the severity of vitamin D deficiency increases. Also, for mothers with moderate-to-severe deficiency, the risk of PROM doubles. Studies on the role of vitamin D and the risk of preterm labor, and the possibility of membrane rupture, are contradictory. Numerous studies have shown an association between vitamin D deficiency and increased

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probability of preterm labor (20). Rupture of the membranes is one of the possible causes of preterm labor due to vitamin D deficiency (16, 21, 22). Maternal vitamin D deficiency increases the risk of preterm labor by approximately 9-fold (23). Vitamin D deficiency during pregnancy and childbirth may play a role in premature rupture of membranes by a mechanism of placental inflammation (24). Hence, Frazipour's study revealed that there was no significant association between serum vitamin D level and premature rupture of membranes (24). The underlying mechanism of the potential protective effect of vitamin D on the risk of preterm labor may be due to the wide variety of immunoregulatory effects of vitamin D.

It seems that vitamin D may protect against preterm labor by reducing infection and inflammation (25), but this has not been confirmed in other studies (26-28). Vitamin D is a known modulator of the immune system (29). It is also an effective stabilizer of amniotic membranes through non-genomic mechanisms (30). It is involved in epigenetic changes through DNA methylation in genes that regulate extracellular matrix regeneration and is effective in maintaining the health of amniotic membranes (31); the health of the amniotic membranes is dependent on the structure of vitamin D (32). Low levels of vitamin D are associated with bacterial vaginosis (33). Therefore, with increasing cases of bacterial vaginosis, premature rupture of the membranes occurs. To the best of our knowledge, there is no report in available sources on the relationship between the severity of vitamin D deficiency and the incidence of PROM. We report for the first time the relationship between vitamin D deficiency severity and the increase in the incidence of PROM.

According to the results of our study, PROM increased the risk of definitive neonatal infection more than four-fold. Inflammation and infection of the chorio decidua refers to the mechanism of premature rupture of the membranes. There is a strong association between premature rupture of membranes and inflammation and intrauterine infections, especially in preterm pregnancies (34). Prolonged rupture of the amniotic sac for more than 18 hours increases the risk of neonatal infection ten-fold. Although the most common complication of prolonged rupture of the membranes is immaturity and its side effects, infection is the most important

preventable complication (35). Women with premature rupture of membranes are highly disposed to chorioamnionitis as a result of increased bacterial colonization of vagina before or after PROM (36). Decreased collagen in embryonic membranes is a predisposing factor for premature rupture of membranes (37).

In the current study, CRP was significantly higher in PROM neonates. It was associated with high sensitivity, specificity and reliability. Increased CRP relates to the pathological confirmation of chorioamnionitis associated with fever (38). CRP is the most common biomarker used for neonatal bacterial sepsis. It is also known as a reliable serum marker for the presence or absence of invasive bacterial infection and antibiotic response in newborns (39). Boonkasediesha showed that neonatal CRP with a cut-off point of 1.90 mg / ml has a very high sensitivity, specificity and positive predictive value (40). In the Boskabadi study, 44% of infants with ruptured membranes had elevated CRP (14).

According to the results of our study, vitamin D deficiency in infants born to PROM mothers also increases the risk of death and developmental delay. The results of a study showed vitamin D deficiency was more pronounced in newborns who died. Vitamin D deficiency may increase the likelihood of death of these infants by increasing respiratory problems and infections (41).

In this study, Apgar scores of neonates with prolonged rupture of membranes were lower than newborns without rupture of membranes. However, this difference was not significant in the first minute Apgar score. Preterm rupture of the membranes is one of the most common pregnancy complications that can affect Apgar score (42). In one study, the duration of the rupture was effective in reducing the Apgar score and increased this risk by 8.5-fold (43). As the interval between rupture of the membranes and delivery increases, the risk of infection in the mother and fetus rises (44). In addition to the increased risk of intrauterine infection, other complications such as placenta abruption, pulmonary hypoplasia, hypoxia, and fetal distress due to umbilical cord compression or prolapse increase (45). A limitation of our study was a lack of placental examination of the infants under study.

Conclusion

Based on the results of this study, maternal and neonatal vitamin D levels and fifth minute Apgar scores were lower in neonates with prolonged rupture of membranes. With increasing severity of vitamin D deficiency, the probability of PROM was higher. Since vitamin D levels in mothers and neonates are correlated with prolonged rupture of membranes, it is recommended that mothers who are at risk for preterm labor receive vitamin D supplementation during pregnancy. It may reduce the incidence of prolonged membrane rupture or prematurity, with consequent reductions in various associated complications.

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Conflict of Interest Statement

The Author(s) declare(s) that there is no conflict of interest.

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TABLES

Table 1: Means of variables of mothers and newborns included in the study

Таблица 1: Среднее значение переменных матерей и новорожденных, включенных в исследование

Variables **Переменные**	**Mean ± Standard deviation** **Среднее значение ± стандартное отклонение**
Mother age (years) Возраст матери (лет)	30.52 ±6.73
Parity Число выношенных беременностей	2.01 ±1.23
Maternal vitamin D levels (mg/dl) Уровни витамина D у матери (мг/дл)	20.66 ±13.03
Neonatal vitamin D levels (mg/dl) Уровни витамина D у новорожденных (мг/дл)	16.17 ±10.78
Gestational age (weeks) Срок беременности (недели)	33.57 ±3.33
First minute Apgar score Оценка по шкале Апгар на первой минуте	7.16 ±2.15
Fifth minute Apgar score Оценка по шкале Апгар на пятой минуте	8.58 ±1.63

Table 2: Comparison of variable means of mothers and neonates with or without prolonged rupture of membranes

Таблица 2: Сравнение средних показателей матерей и новорожденных с длительным разрывом плодных оболочек или без него

Groups **Variables** **Группы** **Переменные**	**Neonates with PROM N=93 (38.6%)** **Новорожденных с ПРПО N=93 (38,6%)**	**Neonates without PROM N=148 (61.4%)** **Новорожденные без ПРПО N=148 (61,4%)**	**Significance level * (T-Test)** **Уровень значимости * (Т-тест)**
Maternal vitamin D levels (mg/ml)	17.29±12.55	22.78±12.92	0.001

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Уровни витамина D у матери (мг/мл)			
Neonatal vitamin D levels (mg/ml) Уровни витамина D у новорожденных (мг/мл)	13.09±8.68	18.21±11.56	0.001
Gestational age (weeks) Срок беременности (недели)	32.88±2.82	33.67±3.40	0.351
first minute Apgar score оценка по шкале Апгар на первой минуте	6.93±2.04	7.29±2.21	0.213
fifth minute Apgar score оценка по шкале Апгар на пятой минуте	8.17±1.74	8.83±1.52	0.003
WBC*10^3 лейкоциты*10^3	16.31±10.90	8.60±4.84	0.059
NRBC	2105.53±5205.55	1325.92±672.93	0.608
ESR СОЭ	41.75±29.84	1.00±0.00	0.102
CRP СРБ	28.21±25.27	16.28±16.63	0.032

The calculations were performed based on standard deviation±mean
Расчеты проводились на основе стандартного отклонения ± среднего

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METADATA_TITLE PAGE

Comparison of vitamin D levels between mothers and infants with and without prolonged membrane rupture

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Keywords: fetal membranes, premature rupture of membranes, infant, newborn, vitamin D, mothers, Apgar score

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