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Original article

Correlation Between Vitamin D Deficiency and Calcium, Phosphorus, Alkaline Phosphatase in Preterm Libyan Infants: Case-Control study

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ABSTRACT

Vitamin D plays a vital role in regulating calcium and phosphorus levels and in bone mineralization. Vitamin D deficiency is a widespread issue contributing to metabolic bone disease in preterm infants. This study aimed to evaluate the biochemical parameters associated with varying levels of vitamin D deficiency. Serum samples were collected from preterm infants at the Neonatal Intensive Care Unit of Althawra Hospital Center, Al Beyda, Libya, from Feb-July 2019. The study included two groups: 62 preterm infants and a control group of 34 full-term infants. Serum levels of calcium, phosphorus, alkaline phosphatase, and 25OH-Vitamin D were measured using enzyme immunoassays and standard method. The study involved 62 preterm neonates, with a median gestational age of 32 weeks (range 28-36) and median birth weight of 1960 gm (range 900-2800 gm). The median levels recorded were calcium at 8.7 mg/dl (p=0.000), phosphorus at 4.1 mg/dl (p=0.584), ALP at 458 U/L (p=0.008), and vitamin D at 13.6 ng/ml. The prevalence of varying degrees of vitamin D deficiency was noted: very severe (9.7% < 5 ng/ml), severe (19.4% 5-10 ng/ml), and overall deficiency (45% 10-20 ng/ml), and suboptimal (25.8% 20-30 ng/ml). Comparing preterm infants to the control group revealed statistically significant differences across all parameters (Vitamin D p=0.000, Ca p=0.007, PO4 p=0.036, and ALP p=0.000). All preterm infants exhibited inadequate vitamin D levels, and there was an inverse relationship between the biochemical parameters and vitamin D deficiency. Notably, the elevated ALP level could serve as a crucial marker for diagnosing vitamin D deficiency in clinical settings.

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INTRODUCTION

It is well recognized that Vitamin D plays a crucial role in regulating calcium and phosphorus balance as well as in the mineralization of bones. Additionally, it has been found to be involved in both the innate immune system and autoimmune responses [1]. A lack of Vitamin D contributes to metabolic bone disease, which is a frequent issue in preterm infants across both developed and developing nations [2,3], and may hinder long-term linear growth [4]. Recent research indicates that subclinical Vitamin D deficiency during infancy, along with premature birth, is linked to severe acute lower respiratory infections that necessitate hospitalization and intensive care [5,6]. Significant calcium

accumulation in the fetal skeleton occurs during the third trimester, putting preterm newborns at a higher risk for osteopenia and fractures [7]. Therefore, ensuring adequate Vitamin D levels in infants, especially among those born prematurely is essential.

The development of the fetal skeleton begins early in pregnancy through the growth and differentiation of cartilage precursors, followed by gradual ossification. This process is carefully regulated by hormones, including growth hormone and parathyroid hormone (PTH), as well as cytokines and vitamins A, D, and C [8]. Most bone mineralization takes place during the third trimester, primarily requiring calcium and phosphorus. The fetus accumulates higher levels of these minerals due to active transport from the mother [9]. Parathyroid hormone-related peptide is thought to be the primary regulator of calcium transport, with PTH also contributing. The active form of vitamin D, 1, 25-dihydroxyvitamin D [1, 25(OH) 2D], which is derived from its precursor 25-hydroxyvitamin D [25(OH) D] [10], facilitates the transfer of calcium across the placenta. The mechanisms of phosphorus transfer are less well understood, but it is believed to involve an active transport process partly mediated by PTH. Ultimately, around 80% of the calcium and phosphorus transfer occurs between the 24th week of gestation and delivery. Consequently, premature infants often do not have sufficient stores of these essential minerals [11].

Vitamin D is a fat-soluble steroid hormone that influences not only bone metabolism but also the optimal functioning of various organ systems [12]. The two main types of vitamin D are ergocalciferol (Vitamin D2) and cholecalciferol (Vitamin D3) [13]. It plays a crucial role in regulating serum calcium, phosphate, and alkaline phosphatase (ALP) levels. Direct measurement of vitamin D levels in the blood is rare [13]; thus, deficiency is often inferred from changes in calcium, phosphate, and ALP levels. This study evaluated these biochemical parameters in individuals with varying degrees of vitamin D deficiency. For effective bone formation, alkaline phosphatase is crucial as it releases phosphate from pyrophosphate to assist in hydroxyapatite deposition. Bones are also a significant reservoir of calcium and phosphorus.

METHODS

Study setting

This research was designed as a case-control study, focusing on serum levels of 25(OH) D in preterm infants, defined as those with a gestational age under 37 weeks, who were born at Althawra hospital center in Al-Beida, Libya. These infants were admitted to the neonatal intensive care unit (NICU) from February to July 2019. We excluded infants with cyanotic heart disease or congenital anomalies. Informed consent was obtained from all participants, and the study was approved by the Institute's Ethics Review Board.

Data collection

A total of 34 apparently healthy full-term infants (both male and female) were selected for this study from the delivery room following normal deliveries; umbilical cord blood samples were collected. Prior to the study, informed consent was secured from all subjects. A direct interview with the mothers collected information through a questionnaire**,** which included variables such as the mode of delivery, vitamin D supplementation, expected delivery date, and additional details from the infants' nursery files, including birth weight, gestational age, and sex. The study comprised two groups: one with 62 preterm infants and a control group of 34 full-term infants, with an analysis of serum vitamin D levels, calcium, alkaline phosphatase, and phosphorus. We conducted Pearson's correlation coefficient measurements to evaluate the relationship of 25(OH) vitamin D levels with gestational age, birth weight, sex, and biochemical parameters.

Vitamin D status assessment

The researchers measured the concentrations of serum calcium, phosphorus, alkaline phosphatase, and 25-OHD vitamin D from umbilical cord blood or venous blood. Vitamin D levels were classified as adequate if the concentration of 25(OH)D was over 30 ng/ml, insufficient if between 20-30 ng/ml, deficient if equal to or below 20 ng/ml, and severe deficiency if less than 10 ng/ml.

Maternal and neonatal clinical data

Maternal data included information on the mode of delivery and pregnancy complications such as gestational diabetes, premature rupture of membranes (PROM), and pre-eclampsia. Whereas, Neonatal data encompassed sex, gestational age, delivery date, expected delivery date, and birth weight. We categorized infants by gestational age into early preterm (<32 weeks), moderate preterm (32–33 weeks), and late preterm (34–36 weeks).

Statistical analysis

The data were analyzed using Minitab version 17, with results expressed as mean ± standard error (mean ± SE). An independent T-Test was performed to compare the control group with data gathered on alkaline phosphatase, calcium, inorganic phosphorus, and vitamin D. One-way ANOVA was utilized to examine variations in vitamin D levels based on age and sex. To assess the relationship between vitamin D and biochemical parameters, Pearson's correlation coefficient was calculated, with a p-value of < 0.05 deemed statistically significant.

RESULTS

In our study, we selected a total of 96 infants, consisting of 62 preterm babies (41 males and 21 females) and 34 full-term control babies (21 males and 13 females). The samples were gathered from February to July 2019 at Althawra Hospital Center in Al-Beida, Libya. Among the preterm infants, the average gestational age was 32 ± 0.269 weeks (ranging from 28 to 36 weeks), and the mean birth weight was 1960 ± 63.3 grams (ranging from 900 to 2800 grams). The antenatal history indicated a rupture of membranes in 29% (n=18) of cases, preterm labour in 53% (n=33), placenta previa in 3% (n=3), and placental abruption in 14% (n=14).

The mean vitamin D level in the preterm group was 13.6 ng/ml, with very severe deficiency (<5 ng/ml) found in 9.7% (n=6), severe deficiency (5-10 ng/ml) in 19.4% (n=12), deficiency (10-20 ng/ml) in 45% (n=28), and suboptimal vitamin D levels (20-30 ng/ml) in 25.8% (n=16). Our analysis revealed no significant correlation between vitamin D deficiency and sex, birth weight, or gestational age. Comparisons of calcium (Ca), inorganic phosphorus (PO4), alkaline phosphatase (ALP), and vitamin D levels between male and female preterm infants showed no significant differences, all with a p-value of 0.05. Additionally, grouping preterm infants by gestational age did not yield significant differences either.

Factors such as gestational age, birth weight, and clinical indicators showed no significant association with vitamin D deficiency among preterm infants. While all preterm infants exhibited Vitamin D deficiency, phosphorus levels averaged 4.1 ± 0.15 mg/dl (not significant at p=0.584), calcium levels averaged 8.7 ± 0.23 mg/dl (highly significant at p=0.00), and alkaline phosphatase levels averaged 435.0 ± 24.1 u/l (also highly significant at p=0.008).

In comparing preterm and full-term infants regarding biochemical parameters (calcium, phosphorus, alkaline phosphatase, and vitamin D), we found significant differences ($p<0.05$).

Measurement of Vitamin D Deficiency in Preterm Infants

The outcomes indicated that all preterm infants exhibited varying degrees of vitamin D deficiency, with 9.7% showing severe deficiency, 19.4% severe deficiency, 45% deficiency, and 25.8% suboptimal levels. Most full-term infants, on the other hand, had optimal vitamin D levels, with only a few demonstrating deficiencies **(**Figure 1).

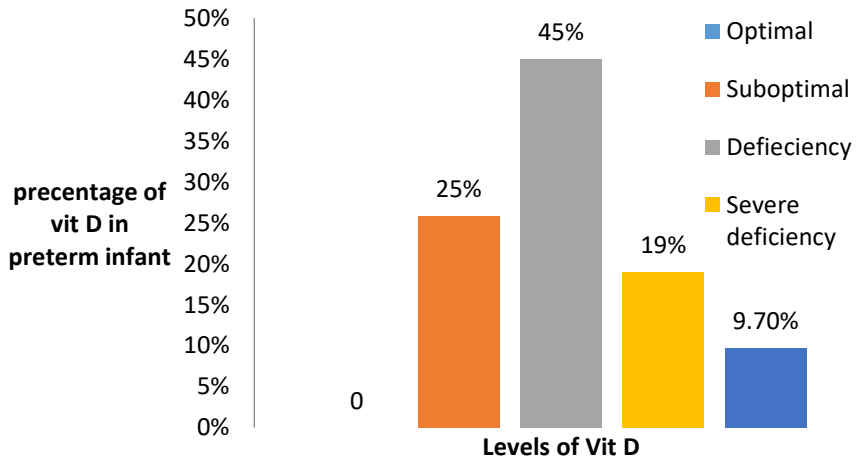

Figure 1. Levels of vitamin D in preterm infants.

Vitamin D and Biochemical Parameters: Comparison between Male and Female Preterm Infants

The serum levels of 25-OHD showed no significant correlation with gender (p=0.373) or other biochemical parameters such as serum calcium (p=0.352), serum phosphorus (p=0.117), and serum alkaline phosphatase (p=0.398) (Table 1).

Table 1. Biochemical parameters studied between male and female preterm infants.

Tests	Female	Male	T-Test
	Mean ±SE	Mean ±SE	
Ca	8.63±0.29	9.08±0.38	0.352
PO4	3.91±0.18	4.48±0.30	0.117
ALP	412±27	459±47	0.398
Vit D	14.03±1.0	12.50±1.4	0.372

Correlation between vitamin D and biochemical parameters in preterm infants

Our research demonstrated an inverse relationship between vitamin D deficiency and calcium levels (p=0.000), and a similar inverse association was found with alkaline phosphatase levels (p=0.008). Conversely, phosphorus levels showed no significant correlation with vitamin D deficiency (p=0.584) (Table 2). Analysis of vitamin D deficiency in full-term infants revealed that only a small number were deficient, while the majority had adequate levels and did not exceed toxicity limits. Specifically, in a group of full-term infants, 5% were deficient in vitamin D, 17% had suboptimal levels, 26% had optimal levels, another 26% were in the upper normal range, and 20% experienced excess without toxicity **(**Figure 2).

Table 2. Correlations between vitamin D and biochemical parameters in preterm, the relationship between vitamin D and biochemical parameters, p-value <0.05 is highly significant.

Parameters	Vitamin D ≤ 20ng/ml	
	R	P-value
Ca	-0.470	0.00
PO4	0.053	0.584
ALP	-0.334	0.008

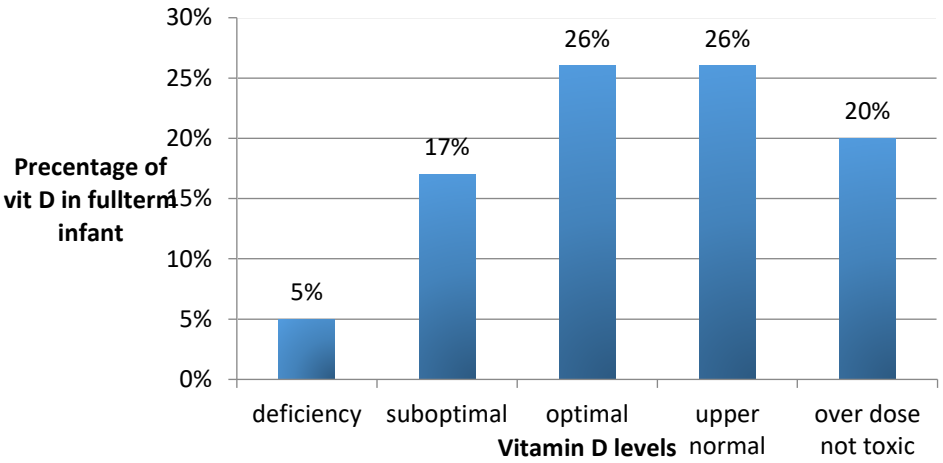

Figure 2 demonstrates levels of Vitamin D in full term infants

Vitamin D status according to birth weight in preterm infants

Vitamin D levels in relation to birth weight in preterm infants showed no significant correlation, with serum 25-OHD concentrations yielding a p-value of 0.618 and a mean birth weight of 1960±63.3 grams (ranging from 900 to 2800 grams) as illustrated in **(**Figure 3).

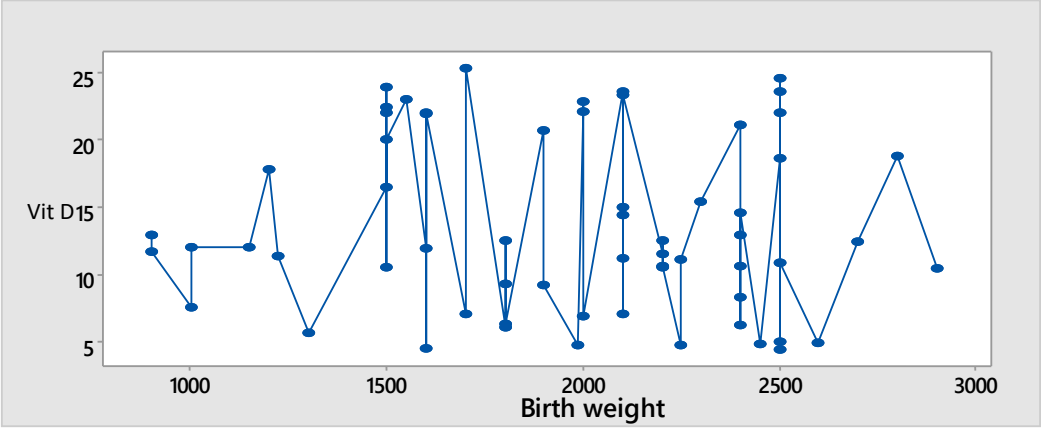

Figure 3. Vitamin D status according to birth weight in pre-term infants.

DISCUSSION

Vitamin D deficiency is prevalent in mothers of preterm infants and in the infants themselves at birth, highlighting a significant public health concern. Routine screening for maternal vitamin D levels and tailored supplementation may enhance vitamin D status and reduce ongoing deficiencies [14]. Numerous studies indicate a high prevalence of vitamin D deficiency in preterm infants, with specific findings such as Monangi, Slaughter [15] reporting staggering deficiency rates across multiple countries. For breastfed infants, vitamin D supplementation is essential since breast milk lacks sufficient vitamin D. The recommended dosage for neonates is 400 IU, although variations exist [16]. Our case-control study involving 96 patients (62 preterm and 34 full-term infants) revealed a vast majority (98.9%) of the preterm group were vitamin D deficient. Further research is required to understand optimally maternal vitamin D levels during pregnancy to ensure proper fetal status.

An Australian study indicated 40% of infants had vitamin D levels below 20ng/ml in cord blood, with a higher likelihood of deficiency linked to younger maternal age and non-White ethnicity [17]. Other studies, including one from Korea [18] found no correlation between vitamin D levels and gestational age or birth weight in preterm infants, which displayed high deficiency rates.

Our findings corroborate that preterm infant face a notably high prevalence of vitamin D deficiency, showing a significant relationship between deficiency and biochemical parameters, while no significant associations were found between deficiency and gestational age, birth weight, or gender. This study also reported no correlation between vitamin D deficiency and delivery mode, mirroring earlier studies [19]. Current evidence does not support vitamin D supplementation solely for preventing preterm birth or preeclampsia; yet low maternal levels of 25-OH D during pregnancy elevate the risks of conditions such as gestational diabetes and preterm labor. Furthermore, our results align with findings from the Korean study [18] showing similar patterns in serum calcium, phosphorus, and vitamin D levels between preterm and full-term infants. Thus, our present report contributes to the ongoing exploration of vitamin D deficiency's impact on maternal health and neonatal outcomes.

Overall, these findings highlight the increased risk of metabolic bone disease in the studied infants who lacked adequate vitamin D and mineral supplementation compared to full-term infants, along with potential risk factors for acute lower respiratory infections. There are currently no established guidelines for vitamin D supplementation for pregnant women in Libya to ensure sufficient vitamin D levels in fetuses and newborns. Additional research is necessary to determine the optimal vitamin D levels for mothers during pregnancy to support adequate fetal vitamin D status, particulary in the Libyan population.

In line with other studies, our findings revealed no correlation between vitamin D deficiency and delivery methods. Instances included premature rupture of membranes (29% mean ± SE 15.5±6.6 in 18 cases), placental abruption (14% mean ± SE 17.5±6.4 in 9 cases), placenta previa (3% mean ± SE 13.4±4.4 in 3 cases), and preterm labor (mean ± SE 11.4±4.4 in 33 cases), as well as maternal complications such as pre-eclampsia. These results echo those from [19], who found no link between vitamin D deficiency and delivery method.

CONCLUSION

To the best of our knowledge, this study represents the first investigation into vitamin D deficiency among preterm infants in Libya, revealing that all preterm infants exhibited insufficient or deficient vitamin D levels. However, this research did not obtain data on maternal vitamin D status during pregnancy, thus preventing an assessment of the

relationship between neonatal vitamin D levels and maternal status. Additionally, the absence of a comparison group of vitamin D-sufficient preterm infants limits our ability to evaluate the impact of vitamin D on neonatal and maternal complications. Further studies involving larger populations of preterm infants are essential to elucidate the intricate interactions of vitamin D. These findings indicate a notable risk of vitamin D deficiency among Libyan newborns, especially in preterm infants.

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Conflicts of Interest
The authors declare that they have no conflict of interest.

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العلاقة بين نقص فيتامين د والكالسيوم والفوسفور والفوسفاتيز القلوية عند الأطفال الليبيين الخدج: دراسة حالة وشاهد في ليبيا (البيضاء)

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المستخلص

يلعب فيتامين د دورًا حيويًا في تنظيم مستويات الكالسيوم والفوسفور وفي تمعدن العظام. يعد نقص فيتامين د مشكلة واسعة النطاق تساهم في أمراض العظام الأيضية عند الأطفال الخدج. تهدف هذه الدراسة إلى تقييم المعايير الكيميائية الحيوية المرتبطة بمستويات متفاوتة من نقص فيتامين د. طُرق تم جمع عينات مصل من الأطفال الخدج في وحدة العناية المركزة لحديثي الولادة بمركز مستشفى الثورة، البيضاء، ليبيا، من فبراير إلى يوليو 2019. شملت الدراسة مجموعتين: 62 طفلًا خديجًا ومجموعة ضابطة مكونة من 34 طفلًا مكتمل النمو. تم قياس مستويات الكالسيوم والفوسفور والفوسفاتيز القلوي (ALP)وفيتامين د 25 OHفي المصل باستخدام اختبارات المناعة الإنزيمية والطرق القياسية. نتائج شملت الدراسة 62 من حديثي الولادة الخدج، بمتوسط عمر حملي 32 أسبوعًا (نطاق 28-36) ومتوسط وزن عند الولادة 1960 جرامًا (نطاق 900-2800 جرام). كانت المستويات المتوسطة المسجلة هي الكالسيوم عند 8.7 مجم / ديسيلتر (قيمة P = 0.000)، والفوسفور عند 4.1 مجم / ديسيلتر (قيمة P = 0.584) ، و ALPعند 458 وحدة / لتر (قيمة P = 0.008) ، وفيتامين د عند 13.6 نانوجرام / مل. لوحظ انتشار درجات متفاوتة من نقص فيتامين د: شديد جدًا (9.7٪ 5 >نانوجرام / مل)، وشديد (19.4٪ 5-10 نانوجرام / مل)، ونقص عام (45٪ 10-20 نانوجرام / مل)، ودون المستوى الأمثل (25.8٪ 20-30 نانوجرام / مل). أظهرت مقارنة الأطفال الخدج بمجموعة التحكم فروقًا ذات دلالة إحصائية عبر جميع المعلمات (فيتامين د ص = 0.000، الكالسيوم ص = 0.007، PO4ص = 0.036، والفوسفاتيز القلوية ص = 0.000). خاتمة أظهر جميع الأطفال الخدج مستويات غير كافية من فيتامين د، وكانت هناك علاقة عكسية بين المعايير الكيميائية الحيوية ونقص فيتامين د. والجدير بالذكر أن مستوى ALP المرتفع يمكن أن يكون بمثابة علامة حاسمة لتشخيص نقص فيتامين د في الإعدادات السريرية.

الكلمات الرئيسية. فيتامين د، التوازن الداخلي، الخدج.