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SERUM VITAMIN D STATUS IN LIBYAN PRETERM BABIES

KEY WORDS:

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ABSTRACT

Objective: Preterm birth (PTB) refer to the delivery of babies before the completion of 37th week of pregnancy and in such children the incidence of vitamin D (vit D) deficiency/insufficiency is studied. The present study is undertaken to bring out the relationship between vitamin D deficiency and its effect on biochemical parameters calcium, phosphorus and alkaline phosphatase in preterm babies and compared with full term babies. **Materials and Methods:** The serum sample from the preterm infants were collected from Neonatal Intensive Care Unit of ALTAWRA HOSPITAL CENTER AL-BAYDA or labor room between February to July 2019. Two groups of infants studied were preterm infants (N=62) and control group full-term infants (N=34). The concentration of serum calcium, phosphorus, alkaline phosphatase and 25OH-Vitamin D from cord blood or venous blood from preterm and full term infants were measured by Enzyme immunoassay and routine methods respectively. **Results:** 62 preterm neonates were taken for the study (median gestational age 32 weeks (28 - 36) weeks, median birth weight 1960 gram (900 – 2800) gram, median calcium 8.7 mg/dl (p=0.000) , median phosphorus 4.1 mg/dl (p=0.584) , median alkaline phosphatase (ALP) 458 U/L (p=0.008) , and median vitamin D level 13.6 ng/ml.) The vitamin deficiency was : Very severe vitamin D deficiency was 9.7 % (< 5 ng/ml) , sever vitamin D deficiency was 19.4 % (5 – 10 ng/ml) , vitamin D deficiency 45 % (10 – 20 ng/ml) suboptimal vitamin D 25.8 % (20 – 30 ng/ml) . When we compare preterm to control (full-term infants) we found the p-value is very highly significantly (p<0.05) in all the parameter vitD (p= 0.00) , ca (p=0.007) , po₄ (p=0.036) and alkaline phosphatase (p=0.00). **Conclusion:** We found that all preterm infant have vitamin D deficiency 99.9 % with no significant relation to gestational age and sex. The correlation between vitamin D deficiency and the biochemical parameters were proportional; when Vit D level is low the ALP level is high (p=0.008) and Ca level is normal or low (p= 0.000).

INTRODUCTION:

Low vitamin D (VitD) status is a risk factor for rickets and has been associated with increased prevalence of respiratory infections and other adverse health outcomes in infants and children. The serum concentration of 25(OH) D is the best indicator of vitamin D status. In contrast to 25(OH) D, circulating 1,25 (OH) 2D is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum concentrations are strictly regulated by parathyroid hormone, calcium, and phosphate. Levels of 1,25(OH)2D do not typically decrease until vitamin D deficiency is severe (27). Vitamin D (Vit D) deficiency has significant health impacts mainly related to calcium and bone metabolism. Significant calcium deposition in the fetal skeleton occurs in utero during the third trimester; thus, preterm infants are at risk of osteopenia (28), which can predispose to fractures (2). Approximately 10-20% of preterm infants <1000g have radio graphically defined rickets (3).¹

To prevent osteopenia of prematurity should be to optimize calcium and phosphorus intake in the majority of cases (4), apart from having normal vitamin D status. In addition to bone and calcium-related issues resulting from severe deficiency, Vit D deficiency has also been related to an increased risk of diabetes mellitus, certain cancers, and autoimmune disease. 5 Apart from Vit D levels, Vit D receptors also do play multiple functions, many of which have not yet been fully elucidated, including roles in regulating genes that are involved in cell growth, immune function, and cardiovascular health. Given the widespread biologic effects of Vit D, it is reasonable to postulate that Vit D deficiency in humans during the fetal and neonatal period may have long-lasting health consequences.

Table 2: As per Institute of Medicine Classification

Classification	
very sever vitamin D deficiency	< 5 ng/ml
sever vitamin D deficiency	5-10 ng/ml

vitamin D deficiency	10-20 ng/ml
Sup optimal vitamin D provision.	20-30 ng/ml
optimal vitamin D level	30-50 ng/ml
upper normal	50-70 ng/ml
overdose but not toxic	70-150 ng/ml
vitamin D intoxication	< 150 ng/ml

Vitamin D supplementation in neonates

Vitamin D supplementation necessary for breastfed infants. Based on available knowledge, breast milk does not contain adequate vitamin D to satisfy infants' requirements. The recommended daily dosage of vitamin D for neonates is 400 units. However, various daily doses of vitamin D, ranging from 100 IU to 1000 IU, have been recommended(29)

Vitamin D and infections

The production of active vitamin D, the active vitamin D binds to VDR and vitamin D responsive elements unlocking DNA, targeting the genes that encode antimicrobial peptides. Vitamin D acts a potent stimulator of antimicrobial peptides, particularly cathelicidin in the human body. It induces the expression of cathelicidin in urogenital epithelial cells, and most importantly the monocyte-macrophage system (30).

Postnatal signs of the infant at risk of vitamin D deficiency

These at-risk infants include:

- babies born at < 37 weeks' gestation
- babies with birth weight < 2 kg
- dark-skinned babies, even if maternal vitamin D levels were healthy in pregnancy
- babies of mothers with known vitamin D deficiency in pregnancy
- Babies of untreated mothers who had been at risk of vitamin D deficiency in pregnancy.

It is worth mentioning that asymptomatic infants at risk for vitamin D deficiency should routinely be started on a vitamin D supplement in the first days of life (29,31)

Signs of vitamin D deficiency in newborns

- Most are asymptomatic
- Cranio-Tabes (softening of skull bones)
- Other osseous signs (such as rickets) are not seen in the newborn, but classical signs in the older infant include the broadening of the metaphyses, bowing of the long bones once weight-bearing and the rachitic rosary (prominent costochondral joints)
- Hypocalcaemia seizures (31, 32)

Aim of the Study:

Our study aimed to find the relationship between vitamin D deficiency and biochemical parameter (calcium, phosphorus ,alkaline phosphatase).To identify the relationship between preterm birth and vitamin D deficiency. To measure vitamin D level in term and preterm infant and compare the levels in the two groups. To study the correlation between vitamin D deficiency levels in neonates at birth and its relationship with gestational age.

MATERIALS AND METHODS:

SUBJECTS:

This project is a case-control study. The preterm infant with gestational age bellow 37 weeks born in the Altawra hospital center al-Bayda and admitted to the neonatal intensive care unit (NICU) between February to July 2019 were included in the study. We excluded children with cyanotic heart disease or congenital anomalies. Informed consent from the participants in the study were taken. Institute's Ethics review Board recommended the study. We have two groups of infants first one preterm infant sixty-two patient and the control group full-term infant thirty-four patient.

Assessment of vit D states:

The concentration of serum calcium, phosphorus, alkaline phosphates and 25-OHD vitamin D we measured from cord blood or venues blood.

Serum levels of vitamin D were considered as adequate when the concentration of 25(OH) D was higher than 30 ng/ml, levels between 20-30 ng/ml were considered as insufficient, and values that were equal or lower than 20 ng/ml defined the diagnosis of vitamin D deficiency and values less than ten ng/ml considered as severe vitamin D deficiency.

Maternal and neonatal clinical data:

Maternal clinical data included mode of delivery and pregnancy complication such as gestational diabetes, premature rupture of membranes (PROM), and pre-eclampsia.

Neonatal clinical data included sex, gestational age, delivery data, expected data, and birth weight. We categorized groups by gestational age as early preterm (<32 weeks of gestation), moderate preterm (32–33 weeks of gestation) and late preterm (34–36 weeks of gestation).

RESULT

All baby collected a total of 96 babies as 62 preterm baby and 34 control full-term baby. In preterm baby, the mean gestational age weeks of study was mean± SE 32±0.269 weeks (28-36 weeks), the mean birth weight was mean± SE 1960±63.3g (900-2800 grams). In ante-natal history rupture membrane rate was 29% (n=18), preterm labor rate was 53% (n=33), placenta previa was 3% (n=3), abruption placenta 14% (n=14).

The mean vit D level was 13.6ng/ml in 62 cases, very severe deficiency vit D was 9.7% (n=6) (□5ng/ml), sever deficiency vit D was 19.4% (n=12) (5-10ng/ml), deficiency vit D was 45% (10-20ng/ml) (n=28) and suboptimal vit D was 25.8% (n=16) (20-30ng/ml).

In the study, there was no correlation between Vat D deficiency and sex, gestational age (week). When we compare between the male and female preterm infant in calcium (cam), inorganic phosphorus(PO4), alkaline phosphatase(ALP) (biochemical parameter) and vitamin D (Vit D) there is no significant different *p-value* =0.05, mean±SE in table 3. And when we divided the preterm infant as group according to gestational age, we found no significant difference as Table 4. The neonatal factor including gestational age, birth weight and clinical factors, were not significantly associated with Vit D deficiency.

In cases preterm infant all have vit D deficiency, the means phosphorus level 4.1±0.15mg/dl was no significant (*p*=0.584), means calcium level 8.70.23mg/dl was highly significant (*p*=0.00), means alkaline phosphatase level 428.224.1u/l was highly significant (*p*=0.008) in table 6.

In the current study, there was a comparison between preterm infant and full-term infant in biochemical parameter (calcium, phosphors , alkaline phosphatase) and vitamin D in separately mean ±SE with different superscript there is high significant different at *p-value* < 0.05 in Table 5

Vitamin D and biochemical parameter comparison between male and female preterm infants

The serum 25-OHD concentration were not significantly correlated to gender (*p*=0.373) , serum ca (*p*=0.352) , serum pho4 (*p*=0.117) , serum alk (*p*=0.398) all not significantly in(Table 3) .

Table 3 Biochemical parameters studied between male and female preterm infants:

	MALE		FEMALE	
	Mean±SE	T-test	Mean±SE	T-test
CA	8.63±0.29	0.352	9.08±0.38	0.352
Po4	3.91±0.18	0.117	4.48±0.30	0.117
ALK	412±27	0.398	459±47	0.398
VIT D	14.03±1.0	0.372	12.50±1.4	0.372

Data are expressed as mean± SE of each sex .within each row for male or female, means without different superscript there

is no significant difference ($p > 0.05$)

Vitamin D status according to gestational age in preterm infants:

The serum 25-OHD concentration were not significantly correlated to gestational age ($p = 0.235$) including delivery mode, small or late for gestational age, maternal PROM, placenta previa in (Fig 4) and in (Table 4).

Vitamin D status according to birth weight in preterm infants:

The serum 25-OHD concentration were not significantly correlated to birth weight ($p = 0.618$) with mean \pm SE {900 – 2800 grams} mean 1960 ± 63.3 grams in (Fig 5).

Table 4 Vitamin D status according to Gestational age (week) in preterm infants:

Age group (week)	Vit D level (mean \pm SD)	p-value
28 week	14.87 \pm 4.49	0.235
29week	11.797 \pm 0.404	
30week	11.40 \pm 3.29	
31week	15.37 \pm 8.62	
32week	13.85 \pm 6.99	
33week	16.78 \pm 7.16	
34week	11.96 \pm 6.13	
35week	10.64 \pm 6.60	
36week	19.93 \pm 7.15	

Data are expressed as mean \pm SE of each age, mean \pm SD without different superscript there is no significant difference ($p > 0.05$).

Vitamin D status and biochemical parameter among full-term and preterm infants: When compared between full-term and preterm we found high significant difference in vitD ($p = 0.000$) and biochemical parameter Ca ($p = 0.007$), ALP ($p = 0.000$), PO₄ ($p = 0.036$). in (Table 5).

Table 5 Comparison of clinical characteristics between infant's full-term and preterm:

Parameter	Full-term		Preterm	
	Mean \pm SE	T-test	Mean \pm SE	T-test
CA	9.68 \pm 0.23	0.007	8.78 \pm 0.23	0.007
ALK	297 \pm 21	0.00	435 \pm 24	0.00
Po ₄	3.67 \pm 0.12	0.036	4.10 \pm 0.16	0.036
VIT D	49.1 \pm 3.8	0.00	13.6 \pm 0.83	0.00

As we comparison between full-term baby and preterm baby separately mean \pm SE with different superscript, there is high significant different at $p > 0.05$.

Correlation between vitamin D and biochemical parameters in preterm infants

The study found that there was an inversely proportional relationship between vitamin D level and calcium since the correlation between them was ($R = -0.47$) and in alkaline phosphatase was ($R = -0.33$). While phosphorus level is directly proportional to vitamin D level ($R = 0.053$). There is no significant difference in serum PO₄ levels between the two groups studied. (0.05) in (Table 6).

Table 6: correlation between vitamin D and biochemical parameters in preterm

Parameter	Vit D ≤ 20 ng/ml	
	R	P-value
CA	-0.470	0.00
PO ₄	0.053	0.584
ALP	-0.334	0.008

The relationship between vit D and biochemical p-value < 0.05 is highly significant.

Measurement of vitamin D deficiency in preterm infants:

We found all the case have differences and not have the average level. Vitamin D level in the preterm infant as a chart

in the vertical axis is a percentage and horizontal axis is vitamin D level.

When we did this study, all of preterm infant have deficiency in difference level of vit D very sever deficiency 9.7%, sever deficiency 19.4%, deficiency 45%, sub optimal 25.8%, and most of full-term have optimal vitamin D within a normal range, and few cases have a deficiency (fig 4).

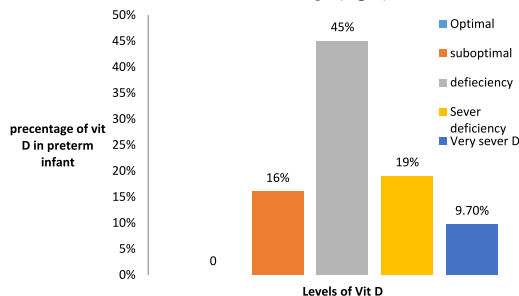
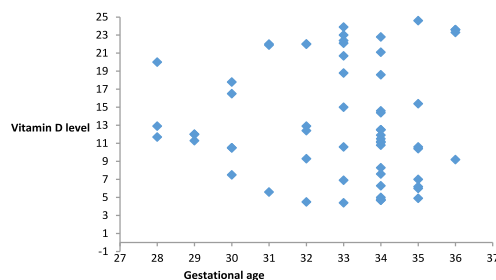
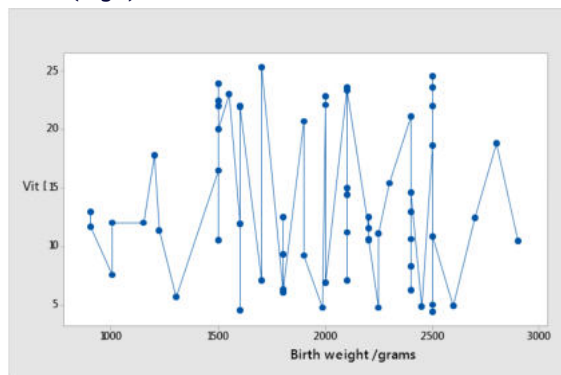


Fig (4): We found most of them deficiency 45%, sever deficiency 19% very severe deficiency 9.7%, and suboptimal 16%. In this study, all preterm infant have vitamin D deficiency.

Vitamin D status according to gestational age in preterm infants (Fig 5):

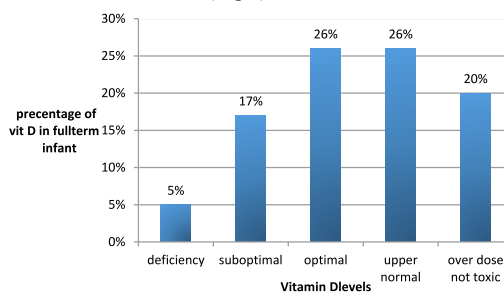


Vitamin D status according to birth weight in preterm infants (Fig 6):



Measurement of vitamin D deficiency in full-term infants:

We found few suffer from vitamin D deficiency, and the majority are normal and have not exceeded the toxicity limit. In control full-term infant has vit D levels have 5% deficiency, 17% suboptimal, 26% optimal, 26% upper normal, 26%, and overdose not toxic 20% (Fig 7).



In present case-control study of 62 cases preterm infants and 34 full-term infant control subjects, the prevalence of vitamin D deficiency in preterm infant is significant and this correlation is noticed in 99.9% of the cases studied 5% of the controls. Multiple observation have shown that preterm babies show vitamin D deficiency .Monangi and slaughter 2014 reported that the rate of vitamin D deficiency in preterm infants in USA is 64%,83% in India, 63.7% in France. A study from Australia reported that vitamin D level in cord blood was 20 ng/ml in 40% of infants. In another study (Park2015), the mean vitamin D level in the premature infant was 16.3 ng/ml, f (vitamin D level was 10.7±6.4 ng/ml in the study of 278 cases with mean gestational age 33±2 weeks)

Some studies have shown that 91% of newborn babies have vitamin D deficiency, and severe vitamin D deficiency is reported to be in 51 %. In our study the rate of vitamin D deficiency in preterm infants is reported to be 99% in 62 premature babies taken for the study with gestational age 28-36 weeks the mean vitamin D level was 13.6 ng/ml. It was observed that very severe vitamin D deficiency was found in 6 cases (9.7 %), critical vitamin D deficiency level in 12 cases (19.4 %) and the rest, vitamin D deficiency (in 28 cases 45%) and sufficiency vitamin D level (in 16 cases 25.8%).

We did not observe any correlation of vitamin D level deficiency and the presence of premature rupture of membrane, abruption placenta, placenta previa. There was no significant statistical correlation between vitamin D level and gender (p=0.398), gestational age (p=0.235) in the premature infant.

Serum 25(OH) D3 is an excellent marker of vitamin D level in the blood. Our result demonstrated that high correlation significant between serum 25(OH) D3 deficiency and serum ALP There was inverse proportional relationship between 25(OH)VitD and ALP 1 (R=0.334) (P-value 0.008), and serum Ca²⁺ a 1 (R=0.470) (P-value 0.000), while, serum levels of phosphate showed non-significant relationship. (R=0.053) (P-value 0.584).

When we comparison between full term infant and preterm infant there is highly significant in means and P-value in full term serum calcium 9.68±0.23mg/dl (P=0.007) preterm serum calcium 8.78±0.23mg/dl (P=0.007) ,full term serum alkaline phosphatase 297±21IU (P=0.00), preterm serum alkaline phosphatase 435±24IU(P=0.00) , full term serum phosphorus 3.67±0.12mg/dl (P=0.036) , preterm serum phosphorus 4.10±0.16mg/dl (P=0.036) , full term serum 25(OH) D3 49.1±3.8ng/ml(P=0.000) , preterm serum 25(OH) D3 13.6±0.83ng/ml (P=0.000).

The recommended vitamin D intake for preterm infants the range should be taking from 150-400 IU/kg/day, many authors opined that with a high intake of calcium and phosphorus is adequate to maintain normal vitamin D levels in healthy preterm infants

CONCLUSION:

In our study, significant vitamin D deficiency 99.9% was found in preterm infants as compared to full-term infants. In this study, the association between biochemical parameter and vitamin D deficiency the high level of ALK and low or normal level of CA on the preterm infants may be a marker of vitamin D deficiency. Vitamin D deficiency in both male and female preterm infants and no significant related to gestational age. More studies are needed with large populations of preterm infants to clarify the complex interactions of vitamin D. A daily dose of vitamin D ranging from 100IU to 1000IU recommended daily dosage of vitamin D for neonates is 400IU/day.

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