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Randomized Controlled Trial Comparing Two Doses of Daily Vitamin D Supplementation in Preterm Neonates

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Abstract--Vitamin D supplementation is recommended for preterm infants. The study aimed to compare the effect of 400 versus 1000 IU of daily oral vitamin D3 doses on vitamin D levels and anthropometric measurements of two groups of newborns. A Randomized controlled trial included eighty successive preterm neonates with a gestational age of ≤ 32 weeks who were randomly allocated to receive 400 or 1000 IU/day of vitamin D. Data were processed using the Statistical Package for Social Science version 23. Vitamin D concentrations at 40 weeks postconceptual age were significantly higher in the 1000 IU (16.26 ± 7.92 ng/ml) when compared to the 400 IU group (12.87 ± 6.15 ng/ml). Serum Calcium showed a highly significant rise in the 1000 IU group when compared with the 400 IU group (9.18 ± 1.76 mg/dL vs. 8.69 ± 0.77 mg/dL, $p < 0.001$). Significant progression in length at follow-up in the 1000 IU group compared with the 400 IU group (42.0 ± 3.45 cm vs 39.93 ± 1.60 cm, $p < 0.001$). *Conclusion: 1000 IU/day of vitamin D supplementation in preterm infants ≤ 32 weeks gestation age effectively decreases the prevalence of vitamin D deficiency and leads to higher concentrations of 25(OH) vitamin D at 40 weeks PCA.*

Keywords---anthropometry, calcium, NICU outcomes, preterm neonates, vitamin D supplementation.

Introduction

Vitamin D is essential in the maintenance of calcium homeostasis. Developmental processes regulated by vitamin D include bone mineralization, lung development, and maturation of the immune system (Maxwell et al., 2012). Vitamin D is mostly transferred to the fetus during the third trimester, so preterm infants are born with lower vitamin D stores with increased needs and lack of sunlight (Hewison, 2011). Breast milk contains inadequate levels of vitamin D, therefore breastfed neonates are almost wholly dependent on supplementation. The American Academy of Pediatrics recommends supplementation of 400 IU of vitamin D per day, whereas the European Society of Gastroenterology, Hepatology, and Nutrition recommends 800–1000 IU per day (Agostoni et al., 2010; Abrams et al., 2013).

The inadequate calcium, phosphorus, and vitamin D stores are exacerbated by inadequate intake, and the high rate of skeletal growth occurring in the first weeks after birth leads to what is known as a metabolic bone disease (MBD). Metabolic bone disease is defined as decreased bone mineral content relative to the expected level of mineralization for a fetus or infant of comparable size or gestational age with biochemical and/or radiographic changes (Rigo et al., 2007; Vachharajani et al., 2009). The prevalence of MBD varies depending on gestational age, birth weight, and kind of alimentation. It occurs in up to 55% of babies born with a weight under 1000 grams and 23% of infants weighing < 1500 grams at birth and it is especially frequent in infants under 28 weeks of gestation (Koo et al., 1989). Common preterm morbidities like bronchopulmonary dysplasia, the use of steroids and calcium wasting diuretics, and the common need for total parenteral nutrition further predispose preterm infants to develop osteopenia (Rigo et al., 2006). Western countries with increased survival of neonates with lower gestational age and birth weight are expected to show a further increase in the frequency of the MBD (Abrams, 2007).

Being low to moderate-income country, the need was urgent to test the two vitamin D supplementation protocols to determine which one would help in minimizing neonatal morbidity and mortality at our neonatal intensive care unit (NICU), with subsequent reduction of the institutional and family burdens related to the long NICU stay. Improvement of the skeletal growth of NICU graduates also adds to a better growth rate in infancy and childhood, which might help to face our growing national problem of stunting and underweight.

Materials and Methods

This study is a randomized clinical trial in which 80 successive preterm neonates who were admitted at the NICU of Obstetrics and Gynecology Cairo Hospital over a period from November 2017 to December 2018. They were randomized via serially numbered opaque sealed envelopes to receive either 400 IU (group A) or 1000 IU (group B) of vitamin D supplementation starting from the time that 100

ml/kg/day of enteral feeding was reached until 40 weeks postconceptional age (PCA). The trial was registered at www.clinicaltrials.gov (NCT03889717) on 25/5/2019.

Patients

Eighty preterm neonates (≤ 32 weeks of gestation) were successively included in the trial, 35 males and 45 females. Fifty-nine were delivered by cesarean section and 21 were delivered vaginally. The gestational age was determined by the first day of the maternal last menstrual period, and confirmed by the neonatologist's physical examination using the Ballard score ([Ballard et al., 1979](#)).

Inclusion criteria

- Gestational age ≤ 32 weeks.
- Birth Weight $\leq 1,500$ grams.

Exclusion criteria

Neonates who had one or more of the following criteria were excluded from the study:

- Major congenital anomalies.
- Chromosomal disorders
- Neonates who died during the follow-up period

Interventions

Anthropometric measurements (weight, length, and skull circumference) were measured within 24 hours after birth and at 40 weeks PCA. The baseline levels of serum calcium, phosphorus, alkaline phosphatase (ALP), and 25-OH vitamin D were measured within 48 hours of age. The neonates included in the trial were randomized using sealed envelopes into one of two groups; Group A received 400 IU/day of vitamin D starting from the day they reached 100 ml/kg/day of enteral feeding until 40 weeks of PCA, and group B received 1000 IU/day of vitamin D starting from the day they reached 100 ml/kg/day of enteral feeding until 40 weeks of PCA. Follow up of serum calcium, phosphorus, alkaline phosphatase, and vitamin D were done after supplementation at 40 weeks PCA.

All neonates enrolled in the study were subjected to the following:

History taking

- Antenatal history including maternal age, parity, educational level, occupation, maternal diseases (SLE, Rheumatic heart disease, epilepsy, diabetes mellitus, hypertension), vaginal bleeding, olig/ polyhydramnios, preterm premature rupture of membranes.
- Natal history including gestational age, birth weight, Apgar score at 1, 5, and 10 minutes and mode of delivery (cesarean section or vaginal delivery) (those data were obtained from pediatrician's record in the operating room).

- Nutritional history including details of total parenteral nutrition (TPN), starting parenteral nutrition and its duration, intravenous calcium administration, time to reach 100 ml/kg/day of enteral feeding, type of enteral feeding, the day newborns started vitamin D supplementation and the duration of supplementation till discharge were all obtained during follow up. Patients who were discharged before completing 40 weeks PCA were followed up after discharge till reaching 40 weeks PCA.
- Complications of prematurity such as bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), feeding intolerance, necrotizing enterocolitis (NEC), sepsis, and final NICU outcome were recorded for each newborn infant.

Clinical examination

- General examination: Assessment of infant well-being through Apgar score (Apgar & James, 1962), was taken from the patient's records. Vital signs were thoroughly recorded and followed all through the ICU stay. Gestational age assessment was confirmed by the neonatologist's clinical examination using the Ballard score (Ballard et al., 1979).
- Anthropometry: weight was recorded using the digital infant scale available in the NICU. The supine length was performed using a hardboard to make a mark on the patient's head and legs then using a tape to measure the distance between both marks to the nearest 0.1cm. Head circumference was measured using a non-stretchable measuring tape with securely wrapping the tape around the widest possible circumference of the head, with the broadest part of the forehead above eyebrow, above the ears from the head side and the most prominent part of the back of the head with measuring the closest 0.1 cm.
- Admission diagnosis: was based on the patient's records. At Kasr Al Aini NICU a diagnosis of RDS based on the clinical presentation on admission, chest x-ray, and venous blood gases.
- Diagnosis of complications: Sepsis was diagnosed by positive blood culture, or, if blood culture results were not available, by a positive C-reactive protein and the immature-to-total neutrophil ratio of more than 0.2 in the presence of clinical signs of sepsis. Sepsis was defined as early-onset if it occurred in the first three postnatal days and late-onset if it occurred after 72 hours of life. Feeding intolerance was diagnosed as the presence of gastric residuals associated with abdominal distension. Diagnosis of necrotizing enterocolitis (NEC) based on Bell's criteria after abdominal x-ray and U/S was done (Cloherty et al., 2008). Diagnosis of IVH based on Volpe's criteria after cranial U/S was done (Tarby & Volpe, 1982). Bronchopulmonary dysplasia (BPD) was diagnosed according to the oxygen requirements at 36 weeks PCA (Cloherty et al., 2008).
- Follow up: patients were followed up after discharge from the NICU till they reached 40 weeks PCA. The vitamin D intake after discharge from NICU was evaluated through questions answered by the mother or caregivers, regarding the use of vitamin D drops, their doses, and compliance.

Laboratory tests

- Complete blood counts, C-reactive protein, and blood culture were obtained.
- Serum calcium (Ca): it was assayed on Beckman Coulter® Au 680, by reacting Arsenazo III with calcium ions to produce an intense purple-colored complex that is measured spectrophotometrically.
- Serum phosphorus (Ph): it was assayed on Beckman Coulter® Au 680, by reacting inorganic phosphate with molybdate to form a heteropoly acid complex that is measured spectrophotometrically.
- Serum alkaline phosphatase (AIP): it was assayed on Beckman Coulter® Au 680. The test reaction is based on the method developed by Bowers and McComb and has been formulated as recommended by the AACC and IFCC.
- 25-OH Vitamin D3: it was measured using a kit manufactured by ORGENTEC Diagnostika GmbH®. The kit employs a competitive enzyme-linked immunosorbent assay (ELISA) method in which 25-OH vitamin D in the sample competes with the 25-OH vitamin D tracer reagent for binding to the 25-OH vitamin D antibodies coated onto the microwells.

Specimen collection

For vitamin D samples: 1 ml whole blood was collected by clean venipuncture from patients after their guardian permission under completely sterile conditions. Blood was allowed to clot for 30 minutes and the serum was separated by centrifugation for 20 minutes at 1000 rpm and preserved in aliquots at -20 °C until used. (All tested serum was clear and non-hemolysed).

- Interpretation of results according to our kit reference (25-OH Vitamin D3/D2, ORGENETIC Diagnostika GmbH, Mainz-Germany):
 - Deficiency : < 12 ng/ml (< 30 nmol/l)
 - Insufficiency : 12 - 20 ng/ml (30 - 50 nmol/l)
 - Sufficiency : > 20 - 150 ng/ml (> 50 - 375 nmol/l)

Vitamin D supplementation

- Vidrop (Vidrop oral drops, Medical Union Pharmaceuticals, MUP, Egypt) was administrated as oral drops (Cholecalciferol 2800 IU/ml).
- Dosing and administration

Each drop is equivalent to 100 IU, so the 1st group (group A) was given 400 IU/Day (4 drops/day) until 40th weeks PCA, while the 2nd group (group B) was given 1000 IU/day (10 drops/day) until 40th PCA.

Statistical analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations, and ranges when parametric and median with inter-quartile range (IQR) when non-parametric. Also, qualitative variables were presented as numbers and percentages. The comparison between groups

regarding qualitative data was done by using the Chi-square test and/or Fisher exact test when the expected count in any cell found less than 5. The comparison between two independent groups with quantitative data and parametric distribution was done by using an Independent t-test while non-parametric distribution was done by using the Mann-Whitney test. The comparison between two paired groups with quantitative data and parametric distribution was done by using a Paired t-test. The comparison between more than two independent groups with quantitative data and parametric distribution was done by using One Way ANOVA. Pearson correlation coefficients test was used to assess the correlation between two quantitative parameters in the same group.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

- P-value > 0.05: Non significant (NS)
- P-value < 0.05: Significant (S)
- P-value < 0.001: Highly significant (HS)

Results

Demographic characteristics

The study included 80 preterm neonates who were admitted to the NICU of the Obstetrics and Gynecology Hospital of Cairo University. They were divided into two groups: A and B. Group A included 40 neonates who received oral vitamin D supplementation at a dose of 400 IU/day, and group B included 40 neonates who received oral vitamin D supplementation at a dose of 1000 IU/ day. Fifty-nine neonates (74%) were delivered by cesarean section, while 21 (26%) were delivered by vaginal delivery with no statistically significant difference between groups A and B, $p=0.204$. There were no statistically significant differences between both groups in gender, gestational age, and birth weight (Table 1). Mothers had a mean age of 26.68 ± 7.65 years for group A and 25.50 ± 4.57 years for group B with no statistically significant difference, $p= 0.407$. None of the mothers received vitamin D supplementation during pregnancy.

Premature rupture of membranes was the most frequent prenatal risk factor ($n=33$, 27.5%), followed by preeclampsia ($n=24$, 30%) and antepartum hemorrhage ($n=18$, 22.5%) with no statistically significant differences between both groups, p value= 0.112, 0.143, 0.592 respectively. Combined respiratory distress syndrome (RDS) and early-onset neonatal sepsis was the most frequent admission diagnosis with no statistically significant difference between both groups (Table 2). There were no statistically significant differences between both groups regarding the gestational age of initiation or duration of vitamin D supplementation (Table 2).

Comparing laboratory outcomes between group A and group B before and after supplementation

Upon initial evaluation, vitamin D insufficiency (defined as levels between 12-20 ng/ml) represented 68.7% of both study groups ($n= 55/80$), followed by deficiency

(levels less than 12 ng/ml) (n=18/80, 22.5%). Only 7 patients of the study population had a sufficient baseline serum vitamin D level (n=7/80, 8.7%). The mean vitamin D level was 12.37 ± 1.03 for ng/ml group A and 12.28 ± 3.22 ng/ml for group B with no statistically significant differences between both groups (Table 3).

Before supplementation, there was no statistically significant difference in the number of patients with deficient, insufficient, and sufficient levels of vitamin D between both groups ($p > 0.05$). After supplementation, there were significantly more deficient patients among group A compared to significantly more sufficient patients among group B ($p = 0.012$, $p = 0.004$ respectively). Although more patients in group A were insufficient in serum vitamin D level after supplementation compared to group B, the difference was not statistically significant ($p > 0.05$) (Table 3), (Fig. 1).

The median (range) serum vitamin D level in patients with sufficient levels of vitamin D was 25 ng/ml (range 21-29) in Group A, while it was 27 ng/ml (range 20-37) among patients in Group B. There was no significant difference regarding follow-up serum level of vitamin D insufficient cases after supplementation between both groups and it didn't reach a level suggestive of toxicity (more than 150 ng/ml) ($p = 1.000$).

There was no statistically significant difference in serum calcium level before vitamin D supplementation between both groups; on the other hand, the serum calcium level was significantly higher after supplementation in group B compared to group A ($p < 0.001$) (Table 3). There was no statistically significant difference in serum phosphorus and alkaline phosphatase levels before and after vitamin D supplementation between both groups (Table 3). There were no statistically significant difference in the mean (\pm SD) serum vitamin D levels before supplementation in both groups, however, the mean serum vitamin D level was significantly higher after supplementation in patients in Group B (16.26 ± 7.92 ng/ml) compared to Group A (12.87 ± 6.15 ng/ml) (Table 3).

Longitudinal follow-up of vitamin D levels in group A before and after supplementation

There was no statistically significant difference regarding the percentage of deficient, insufficient, and sufficient patients according to the serum vitamin D level in neonates of group A before and after supplementation ($p > 0.05$) (Table 4).

Longitudinal follow-up of vitamin D levels in group B before and after supplementation

Among patients in group B who received supplementation with 1000 IU/day of vitamin D, there was a significant decrease in insufficient cases ($p = 0.022$), and a highly significant increase in the sufficient cases after supplementation ($p < 0.001$), (Table 4), (Fig.1).

Follow-up of anthropometric measurements in group A before and after supplementation

There was a statistically significant increase in weight ($p < 0.001$), while there was no statistically significant difference in length and skull circumference before and after vitamin D supplementation in group A ($p > 0.05$) (Table 5).

Follow-up of anthropometric measurements in group B before and after supplementation

There was a significant increase in weight ($p < 0.001$), and a significant increase in length ($p = 0.035$); on the other hand there was no significant difference in skull circumference before and after supplementation in group B ($p > 0.05$) (Table 5).

Duration of vitamin D supplementation

There was no statistically significant difference in the gestational age of initiation or the duration of vitamin D supplementation between both groups (Table 1). There was a statistically significant positive correlation between the duration of vitamin D supplementation and the follow-up serum levels of calcium ($r = 0.752$, $p < 0.001$), phosphorus ($r = 0.567$, $p < 0.001$) and vitamin D ($r = 0.404$, $p < 0.001$) in the patients of both groups (Table 6), (Fig. 2), while there was a statistically significant negative correlation between the duration of vitamin D supplementation and follow-up serum level of alkaline phosphatase in the patients of both groups ($r = -0.501$, $p < 0.001$) (Table 6) (Fig. 3). On the other hand, there was no statistically significant correlation with neither the duration of parenteral nutrition nor the number of days to reach 100 cc/kg/day enteral feeding (Table 6).

Clinical outcomes

There were no statistically significant differences between both groups in the incidence of complications or the duration of respiratory support or NICU stay (Table 2).

Discussion

In our study, the mean baseline vitamin D level on admission was 12.37 ± 1.03 ng/ml in group A and 12.28 ± 3.22 ng/ml in group B, a difference which was not statistically significant ($p > 0.05$) (Table 3). No statistically significant difference was observed between both groups regarding vitamin D sufficiency status at baseline ($p > 0.05$) (Table 3). After supplementation, significantly more patients in group A were deficient compared to group B, and significantly more patients in group B were sufficient compared to group A ($p = 0.012$, $p = 0.004$ respectively) (Table 3).

Moreover, while there was no statistically significant difference regarding the percentage of deficient, insufficient and sufficient patients according to vitamin D level in neonates of group A before and after supplementation ($p > 0.05$), there was a significant decrease in insufficient cases ($p = 0.022$), and a highly significant increase in the sufficient cases after supplementation among patients in group B who received supplementation with 1000 IU/day of vitamin D ($p < 0.001$) (Table 4).

Similar to our study, (Natarajan et al., 2014), reported an improvement in 25(OH) D levels in preterm infants 28-34 weeks when using a target of >20ng/ml in a group supplemented with 800 IU compared to 400 IU. The proportion of infants with vitamin D deficiency was significantly lower in the group which received 800 IU (38.1 %) compared to the group which received 400 IU group at 40 weeks (66.7 %) ($p = 0.01$).

Also, in a study published by (Anderson-Berry et al., 2017), comparing between low (400 IU/day) and high dose (800 IU/day) vitamin D supplementation for 8 weeks in 32 preterm infants with a GA of 24-32 weeks, the group supplemented with 400 IU of vitamin D3 showed that 27% of infants had levels of 25(OH)D3 less than 30 ng/ml as compared to only 11% of infants with 25(OH)D <30 ng/ml in the group supplemented with 800 IU D3. ($p = 0.59$). In their study levels <30ng/ml were considered as deficiency and levels >30ng/ml were considered as vitamin D sufficiency.

Contrary to our findings, (Pittard et al., 1991), supplemented 27 low birth weight preterm infants and 25 full-term infants with either 400 or 800 IU vitamin D from birth to 16 weeks after delivery and obtained similar vitamin D concentrations in both groups. Also, it was found by (Yang et al., 2018), that there was no difference between the high dose (800-1000IU/day) and the low dose (400IU/day) groups in serum levels of calcium, phosphorus, and 25(OH)D concentrations ($p>0.05$).

In our study, there was no significant difference in the frequency of neonatal sepsis ($p>0.05$) or RDS ($p>0.05$) in the patients of both groups (Table 2). This agrees with (Terek et al., 2018), who found no correlation between vitamin D deficiency and neonatal sepsis or respiratory distress. This is also similar to the study by (Onwuneme et al., 2015), who reported no significant statistical correlation between vitamin D level and respiratory distress and sepsis in 94 preterm infants under the gestational age of 32 weeks. But contrary to this are the results of (Fettah et al., 2015), who found a significantly increased respiratory distress risk in infants with vitamin D levels lower than 15 ng/ml.

Our study showed no significant differences between both groups regarding the clinical outcomes like pneumothorax, pulmonary hemorrhage, BPD, NEC, and IVH between the low and the high dose groups ($p>0.05$), (Table 2). Similar to our study are (Terek et al., 2018), in their study on vitamin D deficiency and its effect on neonatal prognosis, they found that the rate of BPD was 25% ($n=7/28$), NEC was 10% ($n=3/28$), IVH was 39% ($n=11/28$). They concluded that there was no correlation between vitamin D deficiency and NEC, BPD, IVH, and mortality.

Contrary to our results, a study by (Yang et al., 2018), conducted among 429 preterm infants to evaluate the effect of vitamin D deficiency on the development of NEC found that the serum 25-OHD levels of preterm infants and their mothers in the NEC group were significantly lower than in the non-NEC group ($P<0.001$). Regarding the duration of respiratory support in our study, there was no significant difference between the studied groups. Contrary to our study (Backström et al., 1999), demonstrated that preterm infants in the lower dose vitamin D supplementation group had a longer duration of assisted ventilation.

Similar to our results are the results of (Bozkurt et al., 2017), in which they conducted a randomized controlled trial (RCT) comparing 3 doses of enteral vitamin D among preterm infants 24-32 weeks; 400IU/day, 800IU/day and 1000 IU/day and found that days on mechanical ventilation ($p=0.2$), on nasal CPAP ($p=0.8$) and oxygen requirement during the entire hospitalization and duration of hospitalization did not differ significantly among the studied groups ($p=0.8$ and $p=0.9$ respectively). The duration of hospital stay between patients of both groups in our study was comparable with no statistically significant difference ($p=0.329$) (Table 2).

An updated meta-analysis was conducted by (Yang et al., 2018), including 12 original randomized controlled studies comparing a low dose (400 IU/day) and a high dose group (800-1000 IU/day) in preterm neonates. In agreement with the current study, they found that length gain was significantly increased in the high dose group ($p<0.05$). On the contrary, they found a statistically significant increase in skull circumference in the high dose group.

These results are in agreement with our findings that vitamin D supplementation of preterm neonates with a dose of 1000IU/day results in a significant increase in length compared to supplementation with a dose of 400IU/day which did not result in a significant increase in length in our study (Table 5). Similar results were found by (Mathur et al., 2016), and (Tergestina et al., 2016), in separate studies. Both studies were conducted in India and compared the effect of giving 400 IU/day and 1000 IU/day of vitamin D3 in preterm infants given over 6 weeks. They concluded that an intake of 1000 IU/day of vitamin D3 is more effective in maintaining vitamin D adequacy and better growth.

Conclusion

Supplementation of preterm neonates with 1000IU/day of vitamin D from the time full enteral feeding is reached resulted in a significant increase in serum vitamin D level at 40 weeks postconceptional age, a significant increase in vitamin D sufficiency status, and a significant increase in length compared to supplementation at a dose of 400 IU/day. A limitation of our study is that it did not include extremely preterm neonates. Confirmation of our findings in extremely preterm neonates is necessary to confirm the benefits and exclude side effects of higher doses of vitamin D in this extremely vulnerable population.

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Compliance with ethical statements

- Conflict of interests: No conflict of interests to declare.
- Funding: There is no funding source.
- Ethical approval: The present work was approved by the ethical committee of the Faculty of Medicine, Cairo University (I-031016).

- Informed consent: An oral and written informed consent was obtained from the parents upon admission to the NICU department for taking samples and using patients' information.

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List of abbreviations

- ALP : alkaline phosphatase
 BPN : bronchopulmonary dysplasia
 Ca : calcium
 d/L : deciliter
 HS : highly significant
 IQR : inter-quartile range
 IU : international unit
 IVH : intraventricular hemorrhage
 MBD : metabolic bone disease
 ml : milliliter
 MUP : Medical Union Pharmaceuticals
 n : number

Ng : nanogram
 NICU : neonatal intensive care unit
 nmol/L: nanomol/liter
 NS : Non-significant
 OH : hydroxy
 PCA : postconceptional age
 Ph : phosphorus
 RDS : respiratory distress syndrome
 Rpm : rate per minute
 S : significant
 SD : standard deviation
 SLE : systemic lupus erythematis
 SPSS : Statistical Package for Social Science
 Statistical Package for Social Science (IBM SPSS)

Author's summary

What is known?

- The importance of vitamin D supplementation for premature neonates once feeding achieved.
- The controversy regarding the proper dosage of vitamin D supplementation in this age group.

What is new?

- Confirmation that a dose higher than routine 400 IU/day is required for premature neonates.
- Finding a relation between vitamin D sufficiency and progression of anthropometric parameters.

Tables and Figures

Table 1
 General characteristics of the study groups

Demographics and interventions	Group A (400 IU/day) n= 40	Group B (1000 IU/day) n = 40	test value	P-value
Gender, n (%)				
Male	21(52.5)	14 (35.0)	2.489	0.115
Female	19(47.5)	26(65.0)		
GA(weeks), mean±SD	30.80 ± 1.07	30.50 ± 1.20	1.183	0.241
APGAR score,				
At 1 min, mean±SD	4.10 ± 1.34	4.05 ± 1.34	0.167	0.868
At 5 min, mean±SD	6.50 ± 1.30	6.58 ± 1.36	0.252	0.801
At 10 min, mean±SD	8.55 ± 0.99	8.65 ± 0.95	0.462	0.645
Admission diagnosis				
Grower	0 (0.0)	1 (2.5)	5.424	0.143
RDS	6 (15.0)	12 (30.0)		

Sepsis	2 (5.0)	0 (0.0)		
RDS & sepsis	32 (80.0)	27 (67.5)		
Surfactant therapy, n (%)				
Yes	9 (22.5)	8 (20.0)	0.075	0.785
No	31 (77.5)	32 (80.0)		
Type of milk, n (%)				
Breast milk	25 (62.5)	19 (47.5)		
Yes	15 (37.5)	21 (52.5)		
No				
Formula	39 (97.5)	38 (95.0)	1.818	0.178
Yes	1 (2.5)	2 (5.0)	0.346	0.556
No			2.464	0.116
Both (breast milk and formula)				
Yes	25 (62.5)	18 (45.0)		
No	15 (37.5)	22 (55.0)		
Days to reach 100 cc/kg/day (days)				
mean±SD	11.47 ± 1.37	10.95 ± 2.14	1.276	0.206
PN, n (%)				
Yes	40 (100.0)	38 (95.0)	2.051	0.152
No	0 (0.0)	2 (5.0)		
Duration of parenteral nutrition (days),				
mean±SD	17.68 ± 4.75	15.47 ± 5.90	1.819	0.073
IV Calcium, n (%)				
Yes	40 (100.0)	37 (92.5)	3.117	0.077
No	0 (0.0)	3 (7.5)		
Start day of vitamin D (days)				
mean±SD	11.29 ± 2.39	12.40 ± 3.42	1.654	0.102
Duration of vitamin D supplementation (days)				
mean±SD	50.87 ± 8.88	54.13 ± 8.78	1.610	0.112
GA when vitamin D started(weeks)				
mean±SD	32.7 ± 1.04	32.63 ± 1.20	0.279	0.781

cm: centimeter; GA: gestational age; IU: international unit; min: minute; n: number; SD: standard deviation; RDS: respiratory distress syndrome; kg: kilogram; PN: parenteral nutrition; IV: intravenous. Data were expressed as mean±standard deviation where the Student-t-test was applied for comparisons or as number and percentage using Chi-square(X^2) test for comparison.

Table 2
Neonatal intensive care outcomes of the study groups

NICU outcomes	Group A (400IU/day) (n = 40)	Group B (1000IU/day) (n = 40)	test value	P-value
Respiratory Support, n (%)				
CPAP	21 (52.5)	24 (60)	0.210	0.648
MV	17 (42.5)	15 (37.5)		
Duration of respiratory support (days),				
mean±SD	16.68±8.10	13.48±9.16	1.656	0.102
Pneumothorax, n (%)				
Yes	8 (20.0)	6 (15.0)	0.346	0.556
No	32 (80.0)	34 (85.0)		
Pulmonary hemorrhage, n (%)				
Yes	2 (5.0)	3 (7.5)	0.213	0.644
No	38 (95.0)	37 (92.5)		

BPD, n (%)				
Yes	4 (10.0)	2 (5.0)	0.721	0.395
No	36 (90.0)	38 (95.0)		
Sepsis, n (%)				
Early				
Yes	30 (75.00)	23(57.50)		
No	10 (25.00)	17(42.50)	2.739	0.098
Late			0.287	0.592
Yes	8(20.00)	10(25.00)		
No	32(80.00)	30(75.00)		
Feeding intolerance, n (%)				
Yes	17 (42.5)	11 (27.5)	1.978	0.16
No	23 (57.5)	29 (72.5)		
NEC, n (%)				
Yes	3 (7.5)	4 (10.0)	0.127	0.692
No	37 (92.5)	36 (90.0)		
IVH, n (%)				
Yes	9(22.5)	8(20.0)	0.075	0.784
No	31(77.5)	32(80.0)		
Duration of hospital stay (days), mean±SD	30.26 ± 8.19	28.08 ± 11.07	0.983	0.329

NICU: neonatal intensive care unit; n: number; CPAP: continuous positive airway pressure; MV: mechanical ventilation; SD: standard deviation; BPD: bronchopulmonary dysplasia; NEC: necrotizing enterocolitis; IVH: intraventricular hemorrhage. Data were expressed as mean±SD where the Student-t-test was applied for comparisons or as number and percentage using Chi-square (X²) test for comparison.

Table 3
Laboratory parameters of the study groups

Laboratory parameters	Group A (400IU/day)	Group B 1000IU/day)	test value	p-value	
Ca(mg/dL), mean±SD					
Before	8.61 ± 0.69	8.58 ± 0.9	0.167	0.868	
After	8.69 ± 0.77	9.18 ± 1.76	3.760	<0.001**	
Ph(mg/dL), mean±SD					
Before	3.98 ± 0.51	3.76 ± 0.59	1.836	0.070	
After	4.1 ± 0.75	3.96 ± 0.61	0.777	0.439	
Alp(IU/L), mean±SD					
Before	244.38 ± 64.44	257.35 ± 66.34	0.887	0.378	
After	237.39 ± 71.14	235.11 ± 48.67	0.164	0.870	
Vitamin D(ng/ml), mean±SD					
Before	12.37 ± 1.03	12.28 ± 3.22	0.235	0.815	
After	12.87 ± 6.15	16.26 ± 7.92	2.098	0.039*	
Baseline Vitamin D,					
Deficient	< 12	11(27.5)	7(17.5)	1.147	0.284
Insufficient	12 – 20	26(65.0)	29(72.5)	0.524	0.469
Sufficient	≥ 20	3(7.5)	4(10.0)	0.157	0.691
Vitamin D at follow- up					
< 12	10(25.0)	2(5.0)	6.275	0.012*	
			0.802	0.370	

Deficient	12-20	23(57.5)	19(47.5)	8.205	0.004*
Insufficient	≥20	7(17.5)	19(47.5)		
Sufficient					

IU: international units; Ca: calcium; mg:milligram; dL: deciliter; Ph: phosphorus; ALP: alkaline phosphatase; ng:nanogram; SD: standard deviation. Data were expressed as mean±SD where student t-test was applied for comparisons or as number and percentage using Chi-square(X^2)test.

Table 4
Follow up of vitamin D sufficiency in each study group before and after supplementation

Group A (400IU/day)		Baseline n (%)	Follow- up n (%)	Test value	P-value
Deficient	< 12	11(27.5)	10(25.0)	0.065	0.798
Insufficient	12 – 20	26(65.0)	23(57.5)	0.474	0.491
Sufficient	≥ 20	3(7.5)	7(17.5)	1.829	0.176
Group B (1000IU/day)		Baseline n (%)	Follow- up n (%)	Test value	P-value
Deficient	< 12	7(17.5)	2(5.0)	3.13	0.076
Insufficient	12 – 20	29(72.5)	19(47.5)	5.208	0.022*
Sufficient	≥ 20	4(10.0)	19(47.5)	13.73	<0.001**

IU: international unit, n: number

Table 5
Follow-up of anthropometric measurements in each study group before and after supplementation

Group A (400IU/day)	Before	After	test value	p-value
Weight (grams), mean±SD	1227.63 ± 153.89	2432.24 ± 240.25	32.844	<0.001**
Length (cm), mean±SD	39.98 ± 2.28	39.93 ± 1.60	0.156	0.877
Skull circumference (cm), mean±SD	29.49 ± 1.23	29.77 ± 0.97	1.586	0.121
Group B (1000IU/day)	Before	After	test value	p-value
Weight (grams), mean±SD	1223.5 ± 148.59	2404.21 ± 272.28	27.938	<0.001**
Length (cm), mean±SD	40.91 ± 2.33	42.0 ± 3.45	2.189	0.035*
Skull circumference (cm), mean±SD	29.06 ± 1.24	29.47 ± 1.47	1.554	0.129

IU: international unit, cm: centimeter, SD: standard deviation

Table 6
Correlations of serum calcium, phosphorus and vitamin D levels of the study groups

Correlations	All patients							
	Follow-up Calcium (mg/dL)		Follow-up Phosphorus (mg/dL)		Follow-up Alp (IU/L)		Follow-up Vitamin D (ng/ml)	
	R	P-value	r	P-value	r	P-value	R	P-value
Duration of vitamin D supplementation (days)	0.752	<0.001**	0.567	<0.001**	-0.501	<0.001**	0.404	<0.001**
Duration of PN (days)	-0.098	0.400	-0.076	0.512	0.110	0.345	0.010	0.935
Days to reach 100 cc/kg/day enteral feeding	-0.084	0.470	-0.071	0.542	0.077	0.509	-0.149	0.200

mg: milligram; dL: deciliter; ALP: alkaline phosphatase; IU: international units; ng: nannogram; PN: parenteral nutrition; kg: kilogram

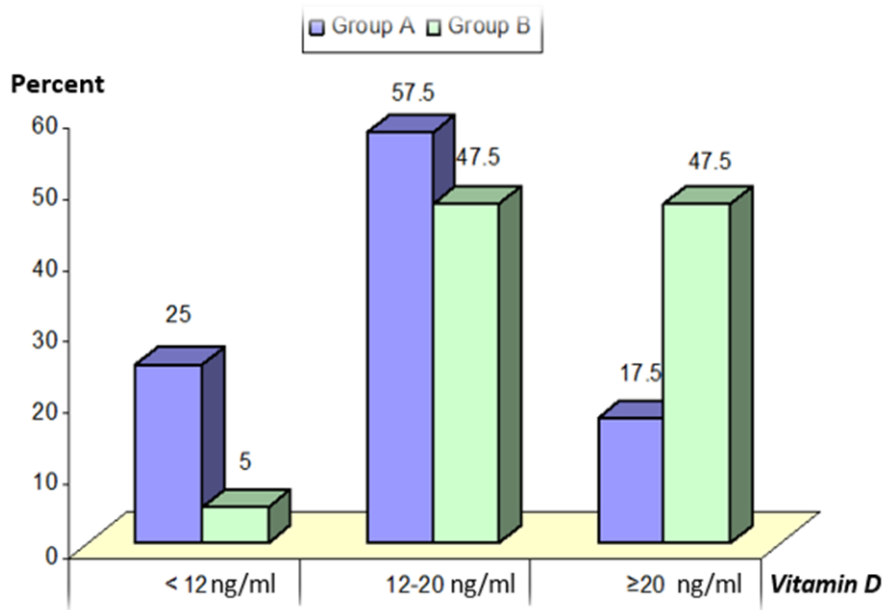


Figure 1. Percentage of vitamin D deficient, insufficient and sufficient after vitamin D supplementation in all cases

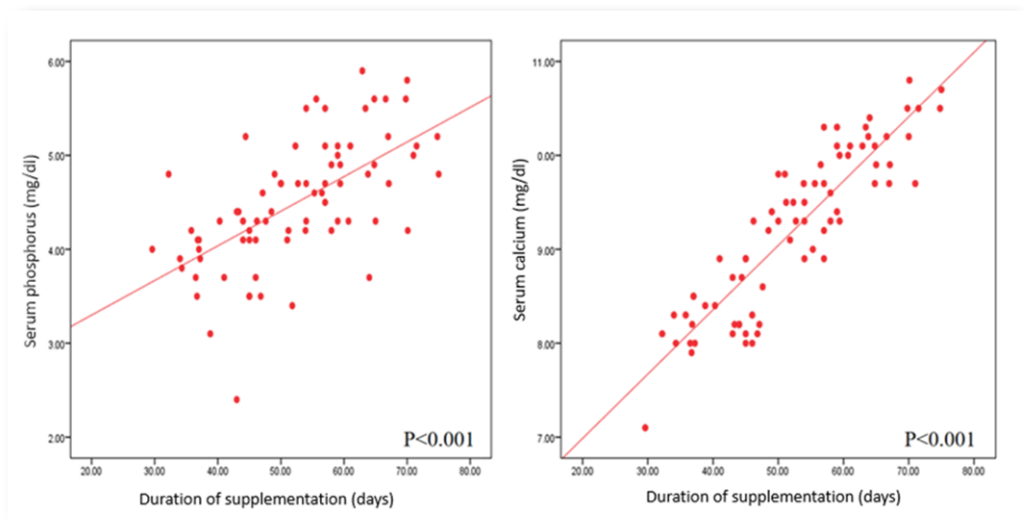


Figure 2. Correlation of duration of vitamin D supplementation to serum calcium and phosphorus levels

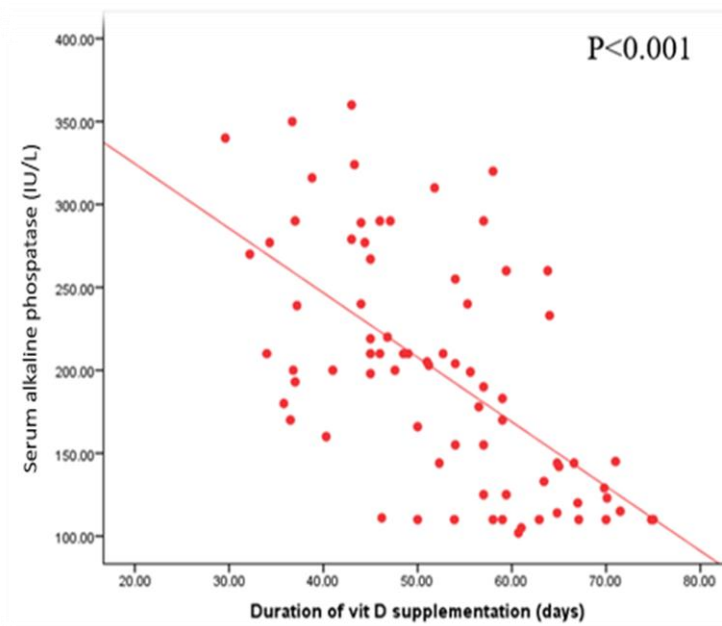


Figure 3. Correlation of the duration of vitamin D supplementation to serum alkaline phosphatase levels