Serum vitamin D deficiency in NICU hospitalized neonates and its association with neonatal outcomes

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Abstract

Background: Neonates could be strongly affected by vitamin D deficiency-related adverse effects. In the present study, we aimed to determine the prevalence of vitamin D deficiency in the neonatal intensive care unit (NICU) hospitalized neonates and its possible relationship with neonatal outcomes.

Methods: A prospective cross-sectional study was carried out in the NICU of Children's Medical Center (Tehran-Iran; 2015). The population study was NICU hospitalized neonates. Immediately on admission, serum sample for vitamin D measurement was obtained with routine blood sampling. Demographic and clinical data including serum levels of vitamin D and calcium, the causes of hospitalization, and neonatal outcome were recorded. The prevalence of vitamin D deficiency and its association with neonatal outcome were assessed.

Results: One-hundred subjects entered the study. Vitamin D deficiency and insufficiency were present in 95% of neonates. There was a significant association between vitamin D status and season of birth (p=0.014); calcium status (p=0.025) and age at NICU admission (p<0.001). The mean value of vitamin D in term neonates was significantly lower than preterm neonates (p=0.031). There were no correlations between neonatal mortality with vitamin D (p=0.876) status.

Conclusion: Vitamin D deficiency/insufficiency was highly prevalent among our NICU hospitalized neonates. There was no association between vitamin D level and neonatal outcomes. Neonates' vitamin D statuses were significantly correlated with the neonate's age at hospital admission, calcium status and season of birth. The measurement of serum vitamin D along with other routine blood tests in neonates who need NICU admission was strongly recommended.

Introduction

Vitamin D as a fat-soluble vitamin plays important roles in calcium and phosphorus homeostasis, bone metabolism, modulating innate and acquired immune responses, inhibition of cancer cell proliferation, regulating organ and hormones functions. Vitamin D receptors (VDR) are expressed in many cells and tissues throughout the body (1, 2). Vitamin D is transferred from the mother to the fetus to provide crucial supports in implantation process, placental formation, and fetal developmental stages by its metabolic, immunomodulatory and anti-inflammatory effects (2, 3).

Several studies have demonstrated a high frequency of vitamin D deficiency among critically ill adults who hospitalized in the intensive care units (ICU) (4–6). An investigation showed that of all critically ill cases, survivors had significantly higher vitamin D concentrations and lower pre-ICU hospital stay compared to non-survivors, however, no association was observed between vitamin D level and mortality (4). Another investigation showed that there was a significant association between vitamin D deficiency and mortality rate in ICU hospitalized adult subjects (5). A high prevalence of vitamin D deficiency was observed among ICU hospitalized severely ill patients; the mean vitamin D concentration in 130 ICU
admitted patients was reported 14.04 ± 6.9 ng/ml. Moreover among the deceased patients, the survival time in the vitamin D deficient cases was shorter than the vitamin D sufficient group (6).

Previous studies have also indicated a high prevalence of vitamin D deficiency among hospitalized critically ill children at intensive care units (7). Significant associations were reported between vitamin D deficiency with the severity of illness, multiple organ dysfunctions, higher admission days and mortality rate in patients admitted to pediatric ICU (8, 9).

The high prevalence rate of vitamin D deficiency was also demonstrated among pregnant women as a high-risk population by 60–80% (10, 11). Moreover, a positive correlation has been demonstrated between mother and neonate's vitamin D levels (12). So it seems that neonates could be strongly affected by vitamin D deficiency-related adverse effects. To the best of our knowledge, there are very few studies assessing the prevalence of vitamin D deficiency among NICU hospitalized neonates and its correlation with mortality/morbidity rate (13–16). In the present study, we aimed to determine the prevalence of vitamin D deficiency in the NICU hospitalized neonates as well as to assess the relationship between vitamin D deficiency and neonatal outcomes.

**Materials And Methods**

**Study design**

A prospective cross-sectional single-center study was carried out in the NICU of Children’s Medical Center affiliated to Tehran University of Medical Sciences (Tehran-Iran) in 2015. The population study was NICU hospitalized neonates. Neonates who received vitamin D supplements or neonates without parents’ written consent were excluded from the study.

Immediately on admission serum sample for vitamin D measurement was obtained with routine blood sampling. Serum vitamin D and calcium levels were measured by Electro-chemiluminescence immunoassay and Calcium Assay kit (colorimetric) methods, respectively. Vitamin D deficiency was defined as serum 25(OH) vitamin D < 10 ng/ml, insufficiency 10–30 ng/ml and sufficiency > 30 ng/ml, respectively (17, 18). Serum Calcium < 8 mg/dL was also considered as hypocalcemia (19). Demographic and clinical data of neonates including age, sex, the season of birth, serum levels of vitamin D and calcium, the causes of hospitalization, and neonatal outcome were recorded. Association between vitamin D levels and neonatal outcome were assessed. Additionally, vitamin D, calcium and demographic characteristic were compared between survivors and non-survivors.

**Sample size**

Based on an investigation by Madden et al. (9); the frequency of vitamin D deficiency was 41%. Using a formula, the proposed sample size of 92, our study had a power of 80% and an alpha error of 0.05.

\[ N = \frac{Z^2 \times P(1-P)}{d^2} = 92 \]
Z = 1.96

P = 0.41

d = 0.1

Ethical considerations

Ethics approval was obtained from the institutional review board of Tehran University of Medical Sciences according to Helsinki declaration. All participants' parents gave written consent before enrollment. Participants' data were considered confidential and no extra cost was imposed on our participants.

Data Analysis

Analyses were statistically performed by using software package SPSS Version 18. Quantitative and qualitative variables were reported by mean ± SD and percent, respectively. Independent Student t, Chi-square, and Bivariate Correlation tests were used for determining associations between vitamin D serum concentration and different variables. The Kaplan-Meier analysis was also performed to analyze the correlations between variables and the neonatal mortality rate. The level of significance was considered as P < 0.05.

Results

One-hundred subjects, 60 females and 40 males entered the study. The mean gestational age and birth weight were 33.19±3.96 weeks and 2758±826.46 g, respectively. Forty-one (41%) neonates were preterm. The causes of hospitalization in 77% were medical and 23% were surgical diseases. The mean age at hospital admission and duration of hospitalization were 12.14±18.51 and 13.09±10 days. Forty-one cases (41%) were born in spring and summer and 59 (59%) were born in fall and winter.

The mean neonatal serum vitamin D level was 14.83±18.96 ng/ml (Min=2, Max=150 ng/ml). These values among hospitalized neonates due to medical and surgical causes were 15.01±19.95 and 14.26±15.61 ng/ml. Vitamin D deficiency and insufficiency were present in 95% of neonates. The mean serum calcium concentration was 8.34±1.25 mg/dL (Min=2, Max=10.8). These values among hospitalized neonates due to medical and surgical causes were 8.27±1.34 and 8.55±0.91 mg/dL. Neonatal hypocalcemia was observed in 35% of cases.

Associations between neonatal vitamin D status with demographic and clinical factors

Demographic and some clinical factors were compared between vitamin D deficient, insufficient and sufficient groups (Table 1). Data analysis indicated a significant association between vitamin D status and season of birth (p=0.014), most of the vitamin D deficient neonates were born in winter. A significant positive association was also found between serum vitamin D and calcium statuses; 54.3% of vitamin D
deficient subjects were also hypocalcaemic (p=0.025). A significant positive relationship was found between age at NICU admission and serum vitamin D status (p<0.001; β=0.714). The mean age at NICU admission in vitamin D sufficient group was significantly higher than the mean age of subjects in deficient or insufficient groups. The mean value of vitamin D in term neonates was significantly lower than preterm neonates (10.74±4.78 ng/ml vs. 20.63±28.10 ng/ml; p=0.031); however, there was no significant difference between the term and preterm neonates regarding the prevalence of vitamin D deficiency, insufficiency and sufficiency (p=0.185). No significant association was found between neonatal sex and the mean level of vitamin D (females; 23.49±3.71 ng/ml vs. males; 15.16±1.97 ng/ml; p=0.305) or with the prevalence's of vitamin D deficiency, insufficiency, and sufficiency (p=0.264). The vitamin D statuses of neonates with underlying diseases are shown in table 2. There were no significant differences in neonates' vitamin D concentrations regarding medical (15.01±19.95 ng/ml) or surgical (14.26±15.61 ng/ml) causes of hospitalization (p=0.869). No significant associations were observed between duration of NICU hospitalization and the prevalences of vitamin D deficiency, insufficiency, and sufficiency (p=0.864).

**Associations between neonatal mortality with demographic and clinical factors**

Of all NICU hospitalized neonates, 5 cases (2 females and 3 males) died. Two cases were preterm. All of them were in the vitamin D deficient and insufficient groups but 2 neonates had hypocalcemia. The survival curve considering vitamin D and serum calcium statuses are shown in Fig. 1 & 2. Kaplan-Meier analysis showed no correlations between neonatal mortality with vitamin D (p=0.876) or calcium (p=0.874) statuses. There were also no significant correlations between neonatal mortality with gestational age or sex (p>0.05). The causes of hospitalization among non-survived cases were medical problems. All neonates with surgical diseases survived during the study period.

**Discussion**

The result of the present study showed that the prevalence of vitamin D deficiency and insufficiency were 37% and 58% in our population study. It delineates that the majority of NICU admitted neonates (95%) had abnormal levels of vitamin D. Consistent with our finding, Tanbakuchi et al. indicated such a high prevalence of vitamin D deficiency/insufficiency (vitamin D<30) in 90% of Iranian high-risk preterm neonates (20). Another study from Iran also showed that 93.3% of 522 newborns had mild to severe vitamin D deficiency at birth (21). Investigations from other countries indicated different prevalence rates of vitamin D deficiency/insufficiency among neonates. For instance, Panda et al. from Australia have demonstrated that 35.7% of NICU admitted preterm neonates had vitamin D insufficiency or deficiency (22). A study from Albany has shown vitamin D deficiency/insufficiency in 80% of NICU admitted preterm infants with birth weight < 1500 gr (23). Other studies from Saudi Arabia and Jordan demonstrated vitamin D deficiency in 86% and 94.1% of newborns (24, 15). These discrepancies in the results may arise from diversities in some influential factors like the mother's type of clothing, geographic region, and sunshine exposure, maternal and neonatal multiple disease states, gestational age, implementing of different laboratory cut-offs, ethnicity, and prenatal intake of vitamin D supplements (11, 25, 26).
The present results showed a significant association between neonates' vitamin D status and neonatal age at hospital admission; younger neonates had lower serum vitamin D levels. This finding may indicate the importance of evaluation of vitamin D status in the neonatal period particularly for NICU hospitalized subjects (27). Panda et al. has also indicated a significant increase in vitamin D levels from the first days of life (57 nmol/L) to 3–4 weeks post-natal period (63.5 nmol/L) among NICU admitted preterm neonates. However, participated subjects in this reported study were prescribed postnatal vitamin D supplements (22).

Our findings showed that neonatal vitamin D status was significantly correlated with the season of birth. Of all vitamin D deficient neonates, the majority of them were born in winter. This association between vitamin D status and season of birth was reported by Maden et al. They showed that neonates who admitted during summer had higher levels of vitamin D compared to those admitted in other seasons. The least levels of vitamin D pertained to subjects who admitted through fall or winter (9). The correlation between vitamin D levels and season of birth was also reported by Korj-Bulos et al. They showed low vitamin D levels in neonates who were born during winter months (15).

Results of the present investigation showed a significant correlation between serum vitamin D and calcium statuses. Consistent with our findings, McNally et al. have demonstrated a positive significant association between vitamin D and calcium levels (28). This finding may confirm the important role of vitamin D in calcium homeostasis. Moreover, it may reveal that in hospitalized newborns, functions of different organs and systems including cardiovascular and hemostasis, immune system, enzymes, and cell receptors may be disturbed by the imbalance of calcium levels resulting in severe medical complications (28).

According to our results, the mean value of vitamin D in term neonates was significantly lower than preterm neonates. Another study from Iran has also indicated a lower level of vitamin D in term neonates compared to the preterm group, however, the difference was not significant (13.39 vs. 13.91 ng/ml; p = 0.850) (27). On the other hand, we could not find any statistically significant difference between term and preterm neonates regarding the prevalence of vitamin D deficiency, insufficiency or sufficiency. It has been reported that there is no significant correlation between gestational age and vitamin D level in infant-mother pairs (27). However, several studies have indicated a greater risk of vitamin D deficiency among preterm infants compared to term infants (5, 29, 30).

Findings of the present study demonstrated no significant differences in neonates' vitamin D concentrations regarding medical or surgical causes of hospitalization. Consistent with our results, Arnson et al. did not also find any relationship between vitamin D levels and the causes of hospitalization (6).

We did not find any association between serum vitamin D statuses with neonatal mortality. Accordance with our findings, Azim et al. reported no association between vitamin D status and mortality rate among hospitalized critically ill patients (4). McNally et al. have shown that the survival of an infant was more associated with the type and severity of underlying complications rather than vitamin D status (28).
Based on the results, no significant association was observed between duration of NICU hospitalization and serum vitamin D status. However, this finding was not confirmed by other studies; another study from Iran has demonstrated a significant relationship between neonatal vitamin D levels with the duration of NICU hospitalization (31). Kim et al. have also reported that the average duration of NICU hospitalization in vitamin D deficient group was significantly longer than vitamin D insufficient or sufficient groups (32).

Our study had several limitations. For instance, the sample size, particularly in vitamin D sufficient group, was so small. The mother's vitamin D levels and its association with neonate's vitamin D status were not assessed because of some shortages in our financial recourse. The associations between neonate's serum vitamin D/calcium levels did not evaluate with some other possible involving factors like parathyroid response and prenatal vitamin D supplementation. Future studies with larger sample sizes and considering more affecting variables are suggested.

**Conclusion**

The results of the present study showed that vitamin D deficiency/insufficiency was highly prevalent among our NICU hospitalized neonates. The vitamin D status was positively correlated with the neonatal age at the hospital admission, calcium status and season of birth. There was no association between vitamin D levels and neonatal outcome. Regarding the high prevalence of abnormal levels of vitamin D in our population study, we highly recommend the measurement of serum vitamin D along with other routine blood tests in neonates who need NICU admission.

**Declarations**

Ethics approval and consent to participate: Ethics approval was obtained from the institutional review board of Tehran University of Medical Sciences according to Helsinki declaration. All participants' parents gave written consent before enrollment.

Consent for publication: Our paper did not include any details related an individual person.

Availability of data and materials: The datasets related our study is available from the corresponding author on reasonable.

Competing interests: The authors declare that there is no conflict of interests.

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Authors' contributions: Dr. ZM and Dr. AHM carried out the design and coordinated the study, participated in most of the experiments. Dr SS and Dr. MM coordinated and carried out all the experiments, Analysis of data and participated in manuscript preparation. All authors have read and approved the content of the manuscript.
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Tables

Table 1: Correlations between vitamin D status and neonatal factors in NICU hospitalized neonates
| P value | Deficient n=37 | Insufficient n=58 | Sufficient n=5 | variables |
|---------|----------------|-------------------|----------------|-----------|
| 0.264   | 26(43.3)       | 31(51.7)          | 3(5)           | Sex [n(%)] |
|         | 11(27.5)       | 27(67.5)          | 2(5)           | Female    |
| 0.185   | 15(36.6)       | 22(53.7)          | 4(9.8)         | Gestational age |
|         | 2(5)           | 2(5)              | 2(5)           | Preterm [n(%)] |
| 0.764   | 27(35.1)       | 46(59.7)          | 4(5.2)         | Causes of hospitalization |
|         | 10(43.5)       | 12(52.2)          | 1(4.3)         | Term [n(%)] |
| 0.014   | 5(13.5)        | 6(10.3)           | 2(40)          | Season of birth [n(%)] |
|         | 4(10.8)        | 24(41.4)          | 0(5.2)         | Spring    |
|         | 13(35.1)       | 16(27.6)          | 2(40)          | Summer    |
|         | 15(40.5)       | 12(20.7)          | 1(20)          | Fall      |
| <0.001  | 6.72±9.54      | 11.09±14.04       | 64.60±33.59    | Winter    |
| 0.864   | 13.81±12.70    | 12.80±7.99        | 11.00±9.82     | Age at admission (days) |
| 0.025   | 18(27.7)       | 44(67.7)          | 3(4.6)         | Duration of hospitalization (days) |
|         | 19(54.3)       | 14(40)            | 2(5.7)         | Calcium status [n(%)] |

Table 2: The vitamin D statuses among neonates with underlying diseases
| Deficient n=37 | Insufficient n=58 | Sufficient n=5 | Variable |
|----------------|-------------------|----------------|----------|
| 3 | 4 | - | Medical causes |
| 6 | 9 | 2 | Hyperbilirubinemia |
| 2 | 3 | - | RDS |
| 5 | 9 | - | Metabolic disorders |
| 1 | 3 | - | Seizure disorders |
| 1 | 4 | - | Sepsis |
| 4 | 2 | - | Electrolyte imbalance |
| 1 | 4 | - | Hypoglycemia |
| 2 | 6 | - | Necrotizing entrocolitis |
| - | - | 1 | Congenital heart diseases |
| 2 | 3 | - | Neonatal diabetes |
| 16 | 7 | 8 | Others |
| 6 | 4 | 2 | surgical causes |
| 1 | - | 1 | Intestinal atresia |
| | | | Myelomeningocele |
| | | | Urologic disorders |

Figures
Figure 1
Figure 2

Comparing survival between vitamin D deficient, insufficient and sufficient groups