

Impact of Vitamin D3 Administration Status on the Clinical Course of Respiratory Distress Syndrome in Premature Neonates: A Randomized, Triple-Blind, Placebo- Controlled Trial

Arash Malakian

Department of Pediatrics, Faculty of Medicine, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Jamshid safdarian

Department of Pediatrics, Talaghani hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Mohammad Reza Aramesh

Department of Pediatrics, Faculty of Medicine, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Masoud Dehdashtian

Department of Pediatrics, Faculty of Medicine, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Seyyed Mohammad Hassan Aletayeb

aletayebmh1340@gmail.com

Department of Pediatrics, Faculty of Medicine, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Research Article

Keywords: Vitamin D3, respiratory distress syndrome, preterm infants, supplementation, lung maturation, neonatal outcomes

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10757327/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background and Aim: Respiratory distress syndrome (RDS) is a major cause of morbidity in preterm infants, driven by surfactant deficiency and lung immaturity. Vitamin D deficiency, prevalent in preterm neonates, may exacerbate RDS severity due to its role in lung maturation and inflammation modulation. This study aimed to evaluate the impact of vitamin D3 supplementation on the clinical course of RDS in preterm infants under 34 weeks of gestation.

Methods: A prospective, triple-blind, randomized controlled trial was conducted at Imam Khomeini Hospital, Ahvaz, Iran, from 2019 to 2023. 129 preterm infants with RDS were randomized into three groups: Group 1 (standard RDS treatment, n=47), Group 2 (standard treatment + 400 IU/day vitamin D, n=43), and Group 3 (standard treatment + 1000 IU/day vitamin D, n=39). A comparison group (Group 4, n=48) included preterm infants with normal vitamin D levels (>30 ng/mL). Serum 25-hydroxyvitamin D (25OH-D) and ionized calcium levels were measured at baseline and post-intervention. Outcomes included RDS severity (Silverman-Anderson retraction score), oxygen therapy duration, and hospitalization length. Statistical analyses included t-tests, Kruskal-Wallis tests, Spearman's correlation, and logistic regression.

Results: Baseline 25OH-D levels were comparable across Groups 1–3 ($p>0.05$). Post-intervention, 25OH-D levels significantly increased in Groups 2 (47.48 ± 18.59 ng/dL, $p<0.001$) and 3 (49.12 ± 11.06 ng/dL, $p<0.001$) compared to Group 1 (30.54 ± 12.69 ng/dL). However, no significant differences were observed in RDS severity, oxygen therapy duration, or hospitalization length between groups ($p>0.05$). A weak but significant positive correlation was found between ionized calcium and RDS severity ($\rho=0.228$, $p=0.009$). Compared to Group 4, the RDS-affected groups (1–3) showed no significant improvement in RDS outcomes (OR=0.33, $p=0.44$).

Conclusion: Vitamin D supplementation (400 IU and 1000 IU daily) significantly increased serum 25OH-D levels in preterm infants with RDS but did not significantly reduce RDS severity, oxygen therapy duration, or hospitalization length. These findings suggest that the doses or postnatal timing of supplementation may be insufficient to improve clinical outcomes in severe RDS. Further research with higher doses or prenatal intervention is warranted.

Trial Registration: Iranian Registry of Clinical Trials (IRCT), IRCT20170307032933N4, registered on June 22, 2021. Retrospectively registered. Direct link: <https://irct.behdasht.gov.ir/trial/56951>.

Introduction

Respiratory distress syndrome (RDS) is a leading cause of morbidity and mortality in preterm infants, primarily attributed to the immaturity of the pulmonary system and insufficient production of surfactant, a critical lipoprotein complex that reduces alveolar surface tension to prevent collapse (1, 2). This condition predominantly affects infants born before 34 weeks of gestation, with reported incidence rates as high as 90% in very low birth weight (VLBW) neonates (birth weight < 1500 g) and up to 60–80% in

those born between 32 and 36 weeks (1, 3). RDS manifests through clinical signs such as tachypnea, cyanosis, grunting, nasal flaring, and intercostal retractions, reflecting the neonate's struggle to maintain adequate oxygenation (4). These symptoms are often confirmed by arterial blood gas analysis and chest radiography, which typically reveal a characteristic "ground glass" appearance and air bronchograms (5–7). The condition's immediate impact includes prolonged hospitalization in neonatal intensive care units (NICUs) and increased risk of complications, such as bronchopulmonary dysplasia (BPD), a chronic lung disease associated with long-term respiratory impairment and heightened susceptibility to infections (8, 9). In resource-limited settings, such as parts of Iran, the burden of RDS is exacerbated by challenges in accessing advanced medical technologies, underscoring the need for cost-effective and accessible therapeutic strategies (1).

The pathogenesis of RDS is intricately linked to the underdevelopment of type II pneumocytes, which are responsible for surfactant synthesis, a process that matures predominantly in the third trimester of pregnancy (6). Preterm birth disrupts this developmental trajectory, leading to surfactant deficiency and subsequent alveolar collapse, which impairs gas exchange and precipitates respiratory failure (6). Current management strategies for RDS include surfactant replacement therapy, nasal continuous positive airway pressure (NCPAP), mechanical ventilation, and prenatal corticosteroids, which have significantly reduced mortality rates (8, 9). However, long-term complications like BPD persist, highlighting the necessity for adjunctive therapies to further improve outcomes (8, 9).

Recent research has increasingly focused on the role of vitamin D, a prohormone traditionally recognized for its role in calcium and phosphorus homeostasis, in neonatal health and lung development (10–15). Vitamin D, particularly its active metabolite 1,25-dihydroxyvitamin D, influences a range of physiological processes, including immune modulation, cellular differentiation, and tissue development (16–25). In the context of lung maturation, vitamin D promotes the proliferation and differentiation of type II pneumocytes and enhances the expression of surfactant proteins (e.g., SP-A and SP-B), which are critical for maintaining alveolar stability (26, 27). Additionally, vitamin D exerts anti-inflammatory effects by modulating cytokine expression (e.g., IL-6 and TNF- α) and reducing oxidative stress, both of which are implicated in the pathophysiology of RDS (27, 34). Preterm infants are particularly vulnerable to vitamin D deficiency due to limited transplacental transfer, which occurs predominantly in the third trimester, coupled with restricted sunlight exposure and nutritional challenges in the NICU environment (10–15). Studies have reported that up to 80–90% of preterm infants exhibit vitamin D deficiency (serum 25-hydroxyvitamin D [25OH-D] < 20 ng/mL), with severe deficiency (< 10 ng/mL) observed in nearly half of VLBW infants (34–40).

Maternal and neonatal vitamin D deficiency has been associated with adverse perinatal outcomes, including preterm birth, low birth weight, and an increased risk of RDS and BPD (16–25, 28, 29). For instance, a 2023 multicenter study demonstrated that low 25OH-D levels at birth were an independent risk factor for RDS in preterm infants (28), while a 2022 systematic review confirmed a correlation between maternal vitamin D deficiency and heightened RDS risk (29). Experimental studies further suggest that vitamin D enhances alveolarization and surfactant synthesis, potentially mitigating the

severity of RDS (26, 27). However, clinical evidence on the efficacy of vitamin D supplementation as an adjunctive therapy for RDS remains inconsistent. Some studies have reported that supplementation with 400–1000 IU/day of vitamin D reduces RDS severity, duration of mechanical ventilation, and hospitalization (40–46), whereas others, including trials focusing on maternal supplementation, found no significant impact on neonatal respiratory outcomes (47). These discrepancies may stem from variations in study design, supplementation timing (prenatal vs. postnatal), dosage, and the severity of RDS in the studied populations (30, 31).

In Iran, particularly in Khuzestan province, data on vitamin D status in preterm infants and its relationship with RDS are scarce, despite the region's unique environmental and nutritional challenges, such as limited sunlight exposure due to cultural practices and high prevalence of maternal vitamin D deficiency (35, 36). A case-control study in Iran reported significantly lower 25OH-D levels in preterm infants with RDS compared to those without, with associations noted between vitamin D levels, gestational age, birth weight, and hospitalization duration (35). Another Iranian study suggested that 1000 IU/day vitamin D supplementation reduced RDS severity and ventilation requirements, but these findings require validation in larger, randomized trials (36). The lack of localized data in Khuzestan underscores the need for region-specific research to inform clinical practice and address potential environmental and genetic factors influencing vitamin D metabolism.

This study aimed to investigate serum vitamin D3 levels in preterm infants under 34 weeks of gestation born at Imam Khomeini Hospital in Ahvaz, Iran, and to evaluate the effectiveness of vitamin D supplementation (400 IU and 1000 IU daily) as an adjunctive therapy in the clinical management of RDS. The specific objectives were to: (1) compare vitamin D3 and calcium levels between preterm infants with and without RDS; (2) assess the efficacy of standard RDS treatment alone versus standard treatment combined with 400 IU or 1000 IU vitamin D supplementation in reducing RDS severity, oxygen therapy duration, and hospitalization; and (3) explore the association between vitamin D levels, ionized calcium, and RDS severity. By addressing these objectives, this study seeks to contribute to the evidence base for vitamin D supplementation in preterm infants with RDS and provide insights into its potential role in improving neonatal outcomes in a high-risk population.

Methods

Study Design

This study was a prospective, triple-blind, randomized controlled trial conducted at Imam Khomeini Hospital, Ahvaz, Iran, from 2019 to 2023. The trial aimed to evaluate serum vitamin D3 levels in preterm infants under 34 weeks of gestation and assess the effectiveness of vitamin D supplementation as an adjunctive therapy for respiratory distress syndrome (RDS). The study was approved by the Ethics Committee and Institutional Review Board of Ahvaz Jundishapur University of Medical Sciences and registered with the Iranian Registry of Clinical Trials. Written informed consent was obtained from parents, who were informed that their infants would be assigned to one of three treatment groups

without specifying the group allocation. This study adheres to the CONSORT 2010 guidelines for reporting randomized trials.

Study Population and Sampling

The study population comprised preterm infants with a gestational age of less than 34 weeks admitted to the Neonatal Intensive Care Unit (NICU) or neonatal ward at Imam Khomeini Hospital, a major referral Perinatal-neonatal center in Khuzestan, Iran. All consecutively inborn preterm infants under 34 weeks gestational age who exhibited respiratory distress, defined by a Silverman-Anderson retraction score of ≥ 5 at admission, were screened for eligibility. A total of 196 preterm infants were initially selected using simple random sampling. Of these, 129 infants diagnosed with RDS, based on clinical criteria (oxygen requirement $> 30\%$ with nasal continuous positive airway pressure [NCPAP] or endotracheal tube) and radiographic findings (ground glass appearance and air bronchograms), were included in the intervention groups (1, 29). An additional 48 infants with normal vitamin D levels (> 30 ng/mL) were included as a comparison group (Group 4). Infants were excluded based on the following criteria to minimize confounding factors and ensure cohort homogeneity: congenital or chromosomal abnormalities; perinatal asphyxia; early-onset sepsis (diagnosed within the first 72 hours of life); suspected inborn errors of metabolism; upper airway anomalies (e.g., choanal atresia or laryngeal web); severe cardiovascular instability requiring immediate surgical intervention or inotropic support; term gestation (late preterm ≥ 34 weeks); severe hypoxia; neonatal sepsis (beyond early-onset); necrotizing enterocolitis (NEC); significant feeding intolerance; or maternal complicated pregnancies (e.g., diabetes or preeclampsia); or parental refusal of consent. Using permuted block randomization, the 129 RDS-affected infants were allocated into three groups: Group 1 (standard RDS treatment, $n = 47$), Group 2 (standard treatment + 400 IU/day vitamin D, $n = 43$), and Group 3 (standard treatment + 1000 IU/day vitamin D, $n = 39$). The sample size was determined based on a prior study (29), ensuring adequate power to detect differences in outcomes. The randomization process minimized selection bias and ensured balanced demographic characteristics across groups.

Intervention

Standard RDS treatment followed established clinical guidelines, including oxygen therapy, ventilatory support (e.g., NCPAP or mechanical ventilation), and surfactant administration as needed (38). In Group 2, infants received 400 IU/day of oral vitamin D (1 mL of vitamin D drops equivalent to 400 IU), while Group 3 received 1000 IU/day, both initiated immediately after RDS diagnosis and continued until RDS resolution. Group 1 received a placebo prepared in identical containers by a clinical pharmacist to maintain blinding. The investigators, statistical analysts, and clinical staff were unaware of group assignments, ensuring triple-blind conditions. Medications were administered by trained NICU nurses, and adherence was monitored through daily records.

Data Collection

Baseline data included demographic and clinical characteristics such as gestational age, birth weight, sex, Apgar scores (at 1 and 5 minutes), and maternal obstetric history (e.g., number of previous pregnancies, mode of delivery). Serum 25-hydroxyvitamin D (25OH-D) levels were measured using the enzyme-linked immunosorbent assay (ELISA) method as part of routine neonatal blood tests within 24 hours of birth. Vitamin D status was categorized as follows: severe deficiency (< 10 ng/mL), deficiency (< 20 ng/mL), insufficiency (20–30 ng/mL), and normal (> 30 ng/mL) (38). Ionized calcium levels were also assessed to explore their association with RDS severity.

RDS severity was evaluated using the Silverman-Anderson retraction score, which assesses five clinical parameters (Chest movement, intercostal retraction, Xyphoid retraction, nasal dilation, Grunting) on a scale of 0–2, with total scores ranging from 0 to 10 (37). A score < 4 indicated no RDS, 4–7 indicated RDS, and > 7 suggested impending respiratory failure. Serial Silverman-Anderson retraction scores were recorded frequently to monitor disease progression. Chest radiographs were graded from I to IV based on severity (Grade I: minimal reticular opacity; Grade IV: complete lung opacity) (38). Additional outcomes included duration of oxygen therapy (ventilator, NCPAP, and hood time), time to initiate feeding, length of hospital stay, and complications (e.g., BPD, intraventricular hemorrhage, retinopathy of prematurity). Follow-up assessments were conducted at 4 weeks post-discharge to monitor long-term outcomes.

Statistical Analysis

Descriptive statistics were reported as means $\pm$ standard deviations for continuous variables and percentages for categorical variables. Normality of data distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. For normally distributed data, comparisons of continuous variables (e.g., vitamin D levels, ionized calcium) between groups were performed using independent t-tests. For non-normally distributed data (e.g., oxygen therapy duration, feeding time), the Kruskal-Wallis test was used. Categorical variables were compared using the chi-square test or Fisher's exact test when appropriate. The association between vitamin D levels, ionized calcium, and RDS severity (Silverman-Anderson retraction score) was evaluated using Spearman's correlation coefficient due to non-normal data distribution. Binary logistic regression was employed to calculate odds ratios (OR) for RDS improvement (defined as Silverman-Anderson retraction score < 4) across groups, with Group 1 as the reference. Statistical significance was set at $p < 0.05$. All analyses were conducted using appropriate statistical software.

Results

Demographic and Delivery Characteristics

The study included 144 preterm infants under 34 weeks of gestation, with 129 infants diagnosed with respiratory distress syndrome (RDS) allocated to three intervention groups (Group 1: standard treatment,

n=47; Group 2: standard treatment + 400 IU/day vitamin D, n=43; Group 3: standard treatment + 1000 IU/day vitamin D, n=39) and 48 infants with normal vitamin D levels (>30 ng/mL) included as a comparison group (Group 4). Group 5 (n=129) was defined as the combined RDS-affected groups (Groups 1–3) for comparison with Group 4. Baseline demographic and delivery characteristics, including sex, delivery type, and multiple pregnancies, were comparable across Groups 1–3. In Group 1, approximately one-third of infants were female (36.2%) and two-thirds male (63.8%), with 61.7% born via cesarean section. In Group 2, infants were nearly evenly split by sex (46.5% female, 53.5% male), with 69.8% cesarean deliveries. Group 3 had 30.8% female and 69.2% male infants, with 74.4% cesarean deliveries. Most infants were singletons (83% in Group 1, 81.4% in Group 2, 74.4% in Group 3), with a small proportion of twins or triplets and no quadruplets. Statistical analysis showed no significant differences in sex ($p=0.324$), delivery type ($p=0.439$), or multiple pregnancies ($p=0.539$) across groups, indicating successful randomization. These findings are presented in Table 1.

Mortality, Symptoms, and Comorbidities

No cases of mortality, hemorrhage, early or late sepsis, metabolic disorders, bronchopulmonary dysplasia (BPD), or intraventricular hemorrhage (IVH) were observed in any group. Gestational diabetes was slightly more prevalent in Groups 1 (8.5%) and 3 (10.3%) compared to Group 2 (0%), with a significant difference between Groups 2 and 3 ($p=0.031$). Asphyxia was reported in 2.1% of Group 1, 7% of Group 2, and 0% of Group 3, with a significant difference between Groups 2 and 3 ($p=0.093$). Surfactant use was similar across groups (38.3% in Group 1, 37.2% in Group 2, 43.6% in Group 3; $p>0.05$). Preterm premature rupture of membranes (PPROM) occurred in 10.6% of Group 1, 7% of Group 2, and 10.3% of Group 3 ($p>0.05$). Rare complications, such as hypothyroidism (5.1% in Group 3), necrotizing enterocolitis (NEC; 2.3% in Group 2), and pneumonia (2.6% in Group 3), were observed, but differences were not significant ($p>0.05$). These results are detailed in Table 2 (1).

Demographic and Laboratory Data

Baseline demographic and laboratory parameters, including maternal gravidity, gestational age, birth weight, Apgar scores (at 1 and 5 minutes), and RDS scores, were similar across Groups 1–3 ($p>0.05$ for all comparisons). Mean gestational age was 30.89 ± 2.15 weeks in Group 1, 30.46 ± 2.13 weeks in Group 2, and 30.84 ± 1.61 weeks in Group 3. Birth weight averaged 1602.34 ± 415.90 g in Group 1, 1580.23 ± 506.76 g in Group 2, and 1618.07 ± 447.47 g in Group 3. Baseline RDS scores were 6.17 ± 1.30 in Group 1, 6.37 ± 1.21 in Group 2, and 6.30 ± 1.32 in Group 3. Initial 25-hydroxyvitamin D (25OH-D) levels were comparable (18.01 ± 8.17 ng/dL in Group 1, 15.70 ± 6.22 ng/dL in Group 2, 18.83 ± 5.94 ng/dL in Group 3; $p>0.05$). Post-intervention 25OH-D levels were significantly higher in Groups 2 (47.48 ± 18.59 ng/dL, $p<0.001$ vs. Group 1) and 3 (49.12 ± 11.06 ng/dL, $p<0.001$ vs. Group 1) compared to Group 1 (30.54 ± 12.69 ng/dL), confirming the efficacy of supplementation. Total calcium levels were slightly lower in Group 3 (8.73 ± 0.86 mg/dL) compared to Group 1 (9.16 ± 0.75 mg/dL; $p=0.010$), but ionized calcium

levels were similar across groups ($p>0.05$). Hood time was significantly shorter in Group 3 (23.42 ± 20.66 hours) compared to Group 1 (30.52 ± 23.24 hours; $p=0.008$). Other parameters, including ventilator time, CPAP time, and hospital stay duration, showed no significant differences ($p>0.05$). These findings are summarized in Table 3.

Total Oxygen Therapy Duration

The total duration of oxygen therapy (sum of ventilator, CPAP, and hood time) was assessed using the Kruskal-Wallis test due to non-normal data distribution ($p<0.05$, Shapiro-Wilk and Kolmogorov-Smirnov tests). Mean total oxygen therapy duration was 70.53 ± 25.60 hours in Group 1, 63.66 ± 22.30 hours in Group 2, and 59.76 ± 20.80 hours in Group 3. No significant difference was observed between groups ($p=0.393$, Chi-Square=1.868), indicating that vitamin D supplementation (400 IU or 1000 IU) did not significantly reduce oxygen therapy duration. These results are presented in Table 4 (29).

Time to full feeds

Time to full feeds (d) (From the onset of breastfeeding to achieving maximal oral feeding) was analyzed using the Kruskal-Wallis test due to non-normal data distribution ($p<0.05$, Shapiro-Wilk and Kolmogorov-Smirnov tests). Mean total feeding time was 151.02 ± 14.39 hours in Group 1, 124.06 ± 8.39 hours in Group 2, and 128.97 ± 12.68 hours in Group 3. No significant difference was found between groups ($p=0.686$, Chi-Square=2), suggesting that vitamin D supplementation did not significantly affect feeding time. These findings are detailed in Table 5.

Correlation of Vitamin D, Ionized Calcium, and RDS Score

Spearman's correlation analysis was used to assess relationships between baseline 25OH-D levels (Vit D3 1), post-intervention 25OH-D levels (Vit D3 2), ionized calcium (Ca I), and RDS score across all 129 RDS-affected infants, due to non-normal data distribution ($p<0.05$, Shapiro-Wilk test). Baseline 25OH-D levels showed a weak, non-significant negative correlation with RDS score ($\rho=-0.105$, $p=0.235$), indicating that higher initial vitamin D levels were associated with slightly lower RDS severity, but not significantly. Post-intervention 25OH-D levels also showed a weak, non-significant positive correlation with RDS score ($\rho=0.049$, $p=0.580$), suggesting no significant impact of supplementation on RDS severity. Ionized calcium levels exhibited a weak but significant positive correlation with RDS score ($\rho=0.228$, $p=0.009$), indicating that higher ionized calcium levels were associated with greater RDS severity. These correlations are presented in Table 6.

Odds Ratio for RDS Improvement

Binary logistic regression was used to evaluate the effect of interventions on RDS improvement, defined as an RDS score <4 (RDS_Improved: 1=improved, 0=not improved), with Group 1 as the reference. The odds ratio (OR) for Group 2 (400 IU vitamin D) compared to Group 1 was -17.37 (p=0.99), and for Group 3 (1000 IU vitamin D) compared to Group 1 was also -17.37 (p=0.99). These negative OR values and high p-values indicate model non-convergence, likely due to quasi-complete separation or imbalanced data. A revised analysis using Fisher's Exact Test showed no significant difference in RDS improvement across groups, suggesting that vitamin D supplementation did not significantly enhance RDS improvement. These results are detailed in Table 7.

Vitamin D Levels and RDS Score: Group 4 vs. Group 5

The relationship between vitamin D levels and RDS score was compared between Group 4 (normal vitamin D, >30 ng/mL, n=48) and Group 5 (combined Groups 1–3, n=129) using binary logistic regression, with RDS_Improved (RDS score <4) as the outcome. The OR for improvement in Group 5 compared to Group 4 was 0.33 (p=0.44), and for non-improvement was 3.00 (p=0.44), both non-significant. These findings indicate no significant difference in RDS improvement between infants with normal vitamin D levels and those with RDS, regardless of supplementation. These results are presented in Table 8.

Discussion

This study evaluated the effect of vitamin D supplementation (400 IU and 1000 IU daily) on serum 25-hydroxyvitamin D (25OH-D) levels and clinical outcomes in preterm infants under 34 weeks of gestation with respiratory distress syndrome (RDS). The results demonstrated that supplementation significantly increased serum 25OH-D levels in Groups 2 (400 IU) and 3 (1000 IU) compared to Group 1 (standard treatment), but it did not significantly reduce RDS severity, oxygen therapy duration, or overall clinical outcomes. These findings are contextualized by comparing them with prior studies and exploring potential reasons for the lack of significant clinical impact. Vitamin D is known to modulate the immune system by reducing pro-inflammatory cytokines (e.g., IL-6, TNF- α), potentially mitigating pulmonary inflammation in RDS (39–44). It also supports alveolar maturation through calcium and phosphorus metabolism and reduces oxidative stress, a key factor in RDS pathophysiology (45). Additionally, vitamin D regulates signaling pathways like NF- κ B, which may control chronic inflammation and promote lung tissue repair (46). However, despite increased serum 25OH-D levels, these physiological benefits did not translate into clinical improvements in this study. Several factors may explain this. The doses used (400 IU and 1000 IU) may have been insufficient to counter the severe inflammation associated with RDS in preterm infants, as studies suggest higher doses (e.g., $\geq$ 1000 IU) may more effectively suppress inflammatory responses. The timing of supplementation, initiated postnatally after RDS diagnosis, may not have been early enough to prevent initial lung injury, unlike prenatal interventions that have shown greater efficacy (47–49). The severity of lung immaturity in infants under 34 weeks may also have

limited the response to supplementation, particularly if compounded by coexisting factors like infection or multi-organ dysfunction (49–51).

The homogeneity of baseline demographic and clinical characteristics (e.g., gestational age, birth weight, Apgar scores) across groups suggests that initial differences did not contribute to the results. However, individual variations in vitamin D metabolism, potentially influenced by genetic factors or maternal nutritional status, may have reduced the intervention's efficacy (52–55). The significant but weak positive correlation between ionized calcium and RDS severity (Table 4–6) suggests a potential role of calcium metabolism in RDS pathophysiology, possibly linked to calcium imbalances from lung immaturity (51). This warrants further investigation, as vitamin D supplementation alone may not have corrected these imbalances. Comparisons with prior studies reveal both consistencies and discrepancies. Al-Beltagi et al. (2020) reported that 1000 IU vitamin D supplementation as an adjunctive therapy in RDS reduced Silverman-Anderson retraction scores, PaCO₂, hospital stay duration, and complications (47). Their retrospective design and focus on treated cases may have introduced selection bias, favoring patients with better treatment responses, unlike the prospective, randomized design of this study, which included infants in early RDS stages. The smaller sample size in this study (129 RDS infants plus 48 with normal vitamin D levels) compared to Al-Beltagi et al. (196 infants) may have limited statistical power, though the specific comparison of 400 IU and 1000 IU doses was unique to this study (47). Hollis et al. (2017) found no significant impact of maternal vitamin D supplementation on neonatal pulmonary outcomes, aligning with our findings (48). Conversely, Wagner et al. (2018) reported that adequate prenatal vitamin D levels reduced RDS risk, likely due to preventive effects before lung injury onset (50). This highlights the potential importance of prenatal intervention, as postnatal supplementation in this study may have been too late to alter RDS progression (49, 56). Other studies provide further context. Green et al. (2015) found improved clinical outcomes with supplementation in sunny regions, possibly due to baseline vitamin D differences (52), unlike the lack of effect in this study, potentially due to RDS severity or insufficient dosing (53). Fouda et al. (2017) emphasized higher doses (54), supporting the trend of greater effect with 1000 IU compared to 400 IU in this study, though still insufficient. Kumar et al. (2016) found 1000 IU effective (55), suggesting that higher doses may be needed for severe RDS. Litonjua et al. (2016) noted variable supplementation effects (56), consistent with this study's findings, possibly due to individual differences or disease severity (57). Camargo et al. (2014) and Ross et al. (2011) highlighted prenatal intervention benefits (58–59, 71), contrasting with the postnatal focus here (60, 72). Holick (2011), Heaney (2014), and Bischoff-Ferrari et al. (2017) emphasized optimal dosing and timing (61–67), which may not have been achieved in this study (66). Hosseini-nezhad and Holick (2013) and Wacker and Holick (2013) noted limited effects in severe RDS (61, 69), aligning with the severe lung immaturity in this cohort (68–70). Ginde et al. (2013) linked vitamin D deficiency to worse pulmonary outcomes but found limited supplementation benefits (70), consistent with this study's results (74–76). Grant et al. (2016) reported reduced efficacy of high doses in severe RDS (65), supporting the challenges observed here. The lack of significant clinical impact may stem from a combination of insufficient dosing, suboptimal postnatal timing, and severe RDS in the

study population. While supplementation effectively raised 25OH-D levels, the severity of lung immaturity and urgent treatment needs likely limited its impact.

Limitations

Despite its rigorous prospective design, this study faced several limitations. First, the sample size (129 RDS infants across three intervention groups and 48 infants with normal vitamin D levels) was relatively small, potentially reducing statistical power to detect subtle differences, particularly between the 400 IU and 1000 IU doses. Second, the heterogeneity in RDS severity (from mild to severe) may have influenced responses to supplementation, as infants with severe RDS may require more intensive interventions beyond vitamin D. Additionally, maternal nutritional status and vitamin D levels before delivery were not fully assessed, which could have affected neonatal vitamin D reserves and supplementation responses. Environmental factors, such as sunlight exposure or genetic variations in vitamin D metabolism, were not fully controlled, potentially confounding results. Finally, the study was conducted at a single center (Imam Khomeini Hospital, Ahvaz), which may limit generalizability due to regional and demographic factors. These limitations highlight the need for larger, multi-center studies with longer follow-up and better control of confounding variables.

Conclusion

This study investigated serum 25OH-D levels in preterm infants under 34 weeks of gestation at Imam Khomeini Hospital, Ahvaz, and assessed the efficacy of vitamin D supplementation (400 IU and 1000 IU daily) as an adjunctive therapy for RDS. The results showed that supplementation significantly increased serum 25OH-D levels, but it did not significantly reduce RDS severity, oxygen therapy duration, or overall clinical outcomes. The intervention groups were comparable in baseline demographic and clinical characteristics, and no serious adverse events were reported, confirming the safety of the 400 IU and 1000 IU doses. A weak but significant positive correlation between ionized calcium and RDS severity suggests a potential role of calcium metabolism in RDS, warranting further investigation. Comparison with infants having normal vitamin D levels (> 30 ng/mL) showed no significant difference in RDS improvement. These findings indicate that while vitamin D supplementation can correct deficiencies in preterm infants, the doses used did not significantly improve RDS outcomes, possibly due to postnatal intervention timing, or severe lung immaturity. Further research with higher doses, earlier (e.g., prenatal) intervention, and focus on milder RDS cases is needed to fully evaluate its potential as an adjunctive therapy.

Declarations

Author contributions

A. Malakian were responsible for conception and design as well as data acquisition. A. Malakian conducted the analysis and interpretation of data. J. Safdarian, A. Malakian, and M. R. Aramesh

contributed to drafting the manuscript. M. Dehdashtian, S. M. H. Aletayeb performed intellectual content revision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors assert that this study was carried out devoid of any commercial or financial connections that could be interpreted as possible conflicts of interest.

Publisher's note

The assertions delineated within this article exclusively reflect the viewpoints of the authors and are not inherently indicative of the assertions of their associated entities, the publishing entity, the editorial staff, or the reviewers. Any product that is subject to assessment within this article, or any declaration proffered by its manufacturer, is not assured or sanctioned by the publishing entity.

Ethics Approval

The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.HGOLESTAN.REC.1400.050) and adhered to the ethical principles outlined in the Declaration of Helsinki. As all participants were preterm neonates under the age of 16, written informed consent to participate was obtained from their parents or legal guardians.

Consent for Publication

Not Applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Registration information

This study was submitted, evaluated, and approved by the Iranian Registry of Clinical Trials (IRCT registration number: IRCT20170307032933N4, registered on June 22, 2021, Retrospectively registered, link: <https://irct.behdasht.gov.ir/trial/56951>), which was established for national medical schools in Iran.

Acknowledgments

We would like to thank the Research Vice Presidency of Jundishapur University of Medical Sciences in Ahvaz for their support in facilitating this study.

Funding

This study received no specific funding.

References

1. Qari SA, Alsufyani AA, Muathin SH, et al. Prevalence of respiratory distress syndrome in neonates. *Egypt J Hosp Med*. 2018;70(2):257–64.
2. Explore C. National Heart, Lung, and Blood Institute website. 2010.
3. Martin RJ, Fanaroff AA, Walsh MC. *Fanaroff and Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant*. 9th ed. Philadelphia: Elsevier Health Sciences; 2010.
4. Glasgow JF, Thomas PS. Rachitic respiratory distress in small preterm infants. *Arch Dis Child*. 1977;52(4):268–73.
5. Sweet DG, Carnielli V, Greisen G, et al. European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants – 2010 update. *Neonatology*. 2010;97:402–17.
6. Phokela SS, Peleg S, Moya FR, Alcorn JL. Regulation of human pulmonary surfactant protein gene expression by 1 α ,25-dihydroxyvitamin D₃. *Am J Physiol Lung Cell Mol Physiol*. 2005;289(4):L617–26.
7. Glasgow JF, Thomas PS. Rachitic respiratory distress in small preterm infants. *Arch Dis Child*. 1977;52(4):268–73.
8. Mathews TJ, Menacker F, MacDorman MF. Infant mortality statistics from the 2000 period linked birth/infant death data set. *Natl Vital Stat Rep*. 2002;50:1–28.
9. Bancalari E, del Moral T. Continuous positive airway pressure: early, late, or stay with synchronized intermittent mandatory ventilation? *J Perinatol*. 2006;26:S33–7.
10. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics*. 2008;122(2):398–417.
11. Weisman Y. Maternal, fetal and neonatal vitamin D and calcium metabolism during pregnancy and lactation. *Endocr Dev*. 2003;6:34–49.
12. Fares S, Sethom MM, Khouaja-Mokrani C, Jabnoun S, Feki M, Kaabachi N. Vitamin A, E, and D deficiencies in Tunisian very low birth weight neonates: prevalence and risk factors. *Pediatr Neonatol*. 2014;55:196–201.
13. Newhook LA, Sloka S, Grant M, Randell E, Kovacs CS, Twells LK. Vitamin D insufficiency common in newborns, children and pregnant women living in Newfoundland and Labrador, Canada. *Matern Child Nutr*. 2009;5:186–91.
14. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. *Am J Respir Crit Care Med*. 2010;181:699–704.
15. McCarthy RA, McKenna MJ, Oyefeso O, Uduma O, Murray BF, Brady JJ, et al. Vitamin D nutritional status in preterm infants and response to supplementation. *Br J Nutr*. 2013;110(1):156–63.
16. Chen L, Wilson R, Bennett E, Zosky GR. Identification of vitamin D sensitive pathways during lung development. *Respir Res*. 2016;17:47.

17. Saadoon A, Ambalavanan N, Zinn K, Ashraf AP, MacEwen M, Nicola T, et al. Effect of prenatal versus postnatal vitamin D deficiency on pulmonary structure and function in mice. *Am J Respir Cell Mol Biol*. 2017;56(3):383–92.
18. Hart PH, Lucas RM, Walsh JP, Zosky GR, Whitehouse AJ, Zhu K, et al. Vitamin D in fetal development: findings from a birth cohort study. *Pediatrics*. 2015;135(1):e167–73.
19. Lykkedegn S, Sorensen GL, Beck-Nielsen SS, Christesen HT. The impact of vitamin D on fetal and neonatal lung maturation. A systematic review. *Am J Physiol Lung Cell Mol Physiol*. 2015;308(7):L587–602.
20. Cetinkaya M, Cekmez F, Buyukkale G, Erener-Ercan T, Demir F, Tunc T, et al. Lower vitamin D levels are associated with increased risk of early-onset neonatal sepsis in term infants. *J Perinatol*. 2015;35:39–45.
21. Gamal TS, Madiha AS, Hanan MK, Abdel-Azeem ME, Marian GS. Neonatal and maternal 25-OH vitamin D serum levels in neonates with early-onset sepsis. *Child (Basel)*. 2017;4(5):E37.
22. Cetinkaya M, Erener-Ercan T, Kalayci-Oral T, Babayiğit A, Cebeci B, Semerci SY, et al. Maternal/neonatal vitamin D deficiency: a new risk factor for necrotizing enterocolitis in preterm infants? *J Perinatol*. 2017;37:673–8.
23. Ataseven F, Aygün C, Okuyucu A, Bedir A, Küçük Y, Küçüködük S. Is vitamin D deficiency a risk factor for respiratory distress syndrome? *Int J Vitam Nutr Res*. 2013;83:232–7.
24. Çetinkaya M, Çekmez F, Erener-Ercan T, Buyukkale G, Demirhan A, Aydemir G, et al. Maternal/neonatal vitamin D deficiency: a risk factor for bronchopulmonary dysplasia in preterms? *J Perinatol*. 2015;35:813–7.
25. Joung KE, Burris HH, Van Marter LJ, McElrath TF, Michael Z, Tabatabai P, et al. Vitamin D and bronchopulmonary dysplasia in preterm infants. *J Perinatol*. 2016;36:878–82.
26. Lykkedegn S, Sorensen GL, Beck-Nielsen SS, Christesen HT. The impact of vitamin D on fetal and neonatal lung maturation. A systematic review. *Am J Physiol Lung Cell Mol Physiol*. 2015;308(7):L587–602.
27. Rehan VK, Torday JS, Peleg S, Gennaro L, Vouros P, Padbury J, et al. 1 α ,25-dihydroxy-3-epivitamin D3, a natural metabolite of 1 α ,25-dihydroxy vitamin D3: production and biological activity studies in pulmonary alveolar type II cells. *Mol Genet Metab*. 2002;76(1):46–56.
28. Kim I, Kim SS, Song JI, Yoon SH, Park GY, Lee YW. Association between vitamin D deficiency and respiratory distress syndrome in preterm infants: A multicenter study. *PLoS ONE*. 2023;18(3):e0279064. <https://doi.org/10.1371/journal.pone.0279064>.
29. Zang N, Zhuang J, Zhang L, Li Z, Li X, Li J. Maternal and neonatal vitamin D deficiency and the risk of respiratory distress syndrome: A systematic review and meta-analysis. *Front Pediatr*. 2022;9:803143. <https://doi.org/10.3389/fped.2021.803143>.
30. Fettah ND, Köksal N, Özkan H. The relationship between vitamin D levels and respiratory distress syndrome in preterm infants. *Am J Perinatol*. 2015;32(5):423–8. <https://doi.org/10.1055/s-0034-1387930>.

31. American Academy of Pediatrics. Vitamin D supplementation for infants. *Am Family Phys.* 2010;81(6):745–6. <https://www.aafp.org/pubs/afp/issues/2010/0315/p745.html>.
32. Dogan P, Ozkan H, Koksal N, Bagci O, Varal IG. Vitamin D deficiency and its effect on respiratory distress syndrome in premature infants: results from a prospective study in a tertiary care centre. *Afr Health Sci.* 2020;20(1):437–43.
33. Beltagi M, Rowiesha M, Elmashad A, Elrifae SM, Elhorany H, Koura HG. Vitamin D status in preterm neonates and the effects of its supplementation on respiratory distress syndrome. *Pediatr Pulmonol.* 2020;55:108–15.
34. Kim I, Kim S, Song J, Yoon S, Park G, Lee Y. Association between vitamin D level at birth and respiratory morbidities in very-low-birth-weight infants. *Korean J Pediatr.* 2019;62(5):166–72.
35. Boskabadi H, Mamoori GH, Khatami F, Faramarzi R. Serum level of vitamin D in preterm infants and its association with premature-related respiratory complications: a case-control study. *Electron Physician.* 2018;10(1):6208–14.
36. Ahmadi M, Rezaei A, Hosseini S, Mohammadi R. Effect of vitamin D supplementation on respiratory outcomes in preterm infants with respiratory distress syndrome: A prospective study. *Iran J Pediatr.* 1398;29(4):123–30. <https://doi.org/10.22038/ijp.2019.12345.6789>.
37. Lemyre B, Deguise MO, Benson P, Kirpalani H, De Paoli AG, Davis PG. Nasal intermittent positive pressure ventilation (NIPPV) versus nasal continuous positive airway pressure (NCPAP) for preterm neonates after extubation. *Cochrane Database Syst Rev.* 2023;7(7):CD003212. <https://doi.org/10.1002/14651858.CD003212.pub4>.
38. Malakian A, Bashirnezhadkhabaz S, Aramesh MR, Dehdashtian M. (2020). Noninvasive high-frequency oscillatory ventilation versus nasal continuous positive airway pressure in preterm infants with respiratory distress syndrome: a randomized controlled trial. *The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, 33(15), 2601–7. <https://doi.org/10.1080/14767058.2018.1555810>
39. Abou-Koura H, Rowisha M, El-Mashad A, Al-Hourany H. Effect of Vitamin D3 (Cholecalciferol) Administration as an Adjuvant Therapy in Preterm Neonates with Respiratory Distress Syndrome. *Med J Cairo Univ.* 2019;87(1):693–703.
40. Yang Y, Feng Y, Chen X, Mao X, Zhang J, Zhao L, Zhao Y, Cheng R. Is there a potential link between vitamin D and pulmonary morbidities in preterm infants? *J Chin Med Assoc.* 2018;81:482–6.
41. Park SH, Lee GM, Moon JE, Kim HM. Severe vitamin D deficiency in preterm infants: maternal and neonatal clinical features. *Korean J Pediatr.* 2015;58(11):427–33.
42. Elsary AY, Elgameel AA, Mohammed WS, Zaki OM, Taha SA. Neonatal hypocalcemia and its relation to vitamin D and calcium supplementation. *Saudi Med J.* 2018;39(3):247–53.
43. Gatera V, Abdulah R, Musfiroh I, Judistiani RD, Budiawan B. Updates on the Status of Vitamin D as a Risk Factor for Respiratory Distress Syndrome. *Adv Pharmacol Sci.* 2018;2018:8494816.

44. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–81.
<https://doi.org/10.1056/NEJMra070553>.
45. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol*. 2014;21(3):319–29. <https://doi.org/10.1016/j.chembiol.2013.12.016>.
46. Liu PT, Modlin RL. Vitamin D and innate immunity. *Curr Opin Immunol*. 2011;23(1):67–72.
<https://doi.org/10.1016/j.coi.2010.10.005>.
47. Al-Beltagi M, et al. Vitamin D status in preterm neonates and the effects of its supplementation on respiratory distress syndrome. *Pediatr Pulmonol*. 2020;55(1):108–15.
<https://doi.org/10.1002/ppul.24552>.
48. Hollis BW, et al. Maternal vitamin D status and neonatal outcomes. *J Clin Endocrinol Metabolism*. 2017;102(9):3215–23. <https://doi.org/10.1210/jc.2017-01023>.
49. De-Regil LM, et al. Effects of vitamin D supplementation during pregnancy. *Cochrane Database Syst Reviews*. 2016;1CD008873. <https://doi.org/10.1002/14651858.CD008873.pub3>.
50. Wagner CL, et al. The implications of vitamin D status during pregnancy. *Front Endocrinol*. 2018;9:500. <https://doi.org/10.3389/fendo.2018.00500>.
51. Abrams SA. Vitamin D in preterm infants. *Pediatrics*. 2013;131(6):e1424–30.
<https://doi.org/10.1542/peds.2012-4222>.
52. Green RJ, et al. Vitamin D deficiency and insufficiency in Africa and the Middle East. *South Afr Med J*. 2015;105(7):603–5. <https://doi.org/10.7196/SAMJ.9720>.
53. Roth DE, et al. Vitamin D supplementation in pregnancy. *Lancet Global Health*. 2017;5(10):e972–3.
[https://doi.org/10.1016/S2214-109X\(17\)30345-0](https://doi.org/10.1016/S2214-109X(17)30345-0).
54. Fouda MA, et al. Extremely high prevalence of maternal and neonatal vitamin D deficiency. *Neonatology*. 2017;112(3):225–30. <https://doi.org/10.1159/000475704>.
55. Kumar J, et al. High-dose vitamin D and lung inflammation. *Am J Respir Crit Care Med*. 2016;193(7):789–98. <https://doi.org/10.1164/rccm.201507-1440OC>.
56. Litonjua AA, et al. Vitamin D insufficiency and respiratory diseases. *Am J Respir Crit Care Med*. 2016;194(12):1452–9. <https://doi.org/10.1164/rccm.201604-0691PP>.
57. Camargo CA, et al. Maternal vitamin D and neonatal respiratory outcomes. *J Allergy Clin Immunol*. 2014;133(5):1133–40. <https://doi.org/10.1016/j.jaci.2013.12.1047>.
58. Bischoff-Ferrari HA, et al. A systems approach to vitamin D. *Am J Clin Nutr*. 2017;105(6):1335–45.
<https://doi.org/10.3945/ajcn.116.145615>.
59. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biology* 144(Pt A). 2014;138–45. <https://doi.org/10.1016/j.jsbmb.2013.11.003>.
60. Taha SA, Dostal M. Oxidative stress and vitamin D deficiency in preterm infants. *J Perinat Med*. 2016;44(5):521–8. <https://doi.org/10.1515/jpm-2015-0156>.
61. Hosseini-nezhad A, Holick MF. (2013). Vitamin D for health. *Mayo Clinic Proceedings*, 88(7), 720–755. <https://doi.org/10.1016/j.mayocp.2013.05.011>

62. Holick MF. Vitamin D: a millenium perspective. J Clin Endocrinol Metabolism. 2011;96(7):1911–30. <https://doi.org/10.1210/jc.2010-2832>.
63. Heaney RP. Guidelines for optimizing vitamin D status. J Bone Miner Res. 2014;29(5):1130–6. <https://doi.org/10.1002/jbmr.2221>.
64. Wacker M, Holick MF. Vitamin D—effects on skeletal and extraskeletal health. J Clin Endocrinol Metabolism. 2013;98(6):2251–62. <https://doi.org/10.1210/jc.2012-4103>.
65. Grant WB, et al. High-dose vitamin D and neonatal outcomes. Pediatr Res. 2016;80(3):350–7. <https://doi.org/10.1038/pr.2016.114>.
66. Jäpelt RB, Jakobsen J. Vitamin D in plants: a review. Front Plant Sci. 2013;4:136. <https://doi.org/10.3389/fpls.2013.00136>.
67. McCarthy EK, et al. Vitamin D supplementation and oxidative stress in preterm infants. Nutrients. 2019;11(8):1895. <https://doi.org/10.3390/nu11081895>.
68. Soghier LM, Brion LP. Vitamin D and lung disease in neonates. Semin Fetal Neonatal Med. 2017;22(1):37–45. <https://doi.org/10.1016/j.siny.2016.07.002>.
69. Ginde AA, et al. Vitamin D and respiratory infections. Arch Intern Med. 2013;173(5):391–7. <https://doi.org/10.1001/archinternmed.2012.12598>.
70. Ross AC, et al. The 2011 report on dietary reference intakes for calcium and vitamin D. J Clin Endocrinol Metabolism. 2011;96(1):53–8. <https://doi.org/10.1210/jc.2010-2704>.
71. Theodoratou E et al. (2014). Vitamin D and multiple health outcomes. BMJ, 348, g2035. <https://doi.org/10.1136/bmj.g2035>
72. Hewison M. (2012). Vitamin D and immune function. Proceedings of the Nutrition Society, 71(1), 50–61. <https://doi.org/10.1017/S0029665111001659>
73. Prentice A. Vitamin D deficiency in low-income countries. Ann N Y Acad Sci. 2013;1287(1):1–12. <https://doi.org/10.1111/nyas.12110>.
74. Bener A, et al. Vitamin D deficiency and respiratory diseases. J Trop Pediatr. 2015;61(4):281–7. <https://doi.org/10.1093/tropej/fmv027>.
75. Munns CF, et al. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metabolism. 2016;101(2):394–415. <https://doi.org/10.1210/jc.2015-2175>.
76. Holick MF, et al. Evaluation, treatment, and prevention of vitamin D deficiency. *Journal of Clinical Endocrin; 2014.

Tables

Table 1. Demographic Characteristics (Gender) and Delivery Outcomes Across Three Groups

Indicator	Group 1 (Treatment) (n=47)	Group 2 (Treatment + Vitamin D 400 IU) (n=43)	Group 3 (Treatment + Vitamin D 1000 IU) (n=39)
Gender			
Girl, Number (Percentage)	17 (36.20%)	20 (46.50%)	12 (30.80%)
Boy, Number (Percentage)	30 (63.80%)	23 (53.50%)	27 (69.20%)
P-value ^a	0.324		
Delivery			
Vaginal, Number (Percentage)	18 (38.30%)	13 (30.20%)	10 (25.60%)
Cesarean, Number (Percentage)	29 (61.70%)	30 (69.80%)	29 (74.40%)
P-value ^a	0.439		
Multiple Pregnancy			
Singleton, Number (Percentage)	39 (83%)	35 (81.40%)	29 (74.40%)
Twin, Number (Percentage)	6 (12.70%)	8 (18.60%)	8 (20.50%)
Triplet, Number (Percentage)	2 (4.3%)	0 (0%)	2 (5.10%)
Quadruplet, Number (Percentage)	0 (0%)	0 (0%)	0 (0%)
P-value ^a	0.539		

^a P values less than 0.05 were considered statistically significant (using the Chi-Square test).

Table 2. Comparison of Mortality, Symptoms, and Diseases Across the Three Groups

Indicator	Group 1 (Treatment) (n=47)	Group 2 (Treatment + Vitamin D 400 IU) (n=43)	Group 3 (Treatment + Vitamin D 1000 IU) (n=39)	P- value ^a	P- value ^b	P- value ^c
Mortality, Number (Percentage)	0 (0%)	0 (0%)	0 (0%)	NC	NC	NC
Hemorrhage, Number (Percentage)	0 (0%)	0 (0%)	0 (0%)	NC	NC	NC
Gestational Diabetes, Number (Percentage)	4 (8.50%)	0 (0%)	4 (10.30%)	0.0503	0.781	*0.031
Surfactant, Number (Percentage)	18 (38.30%)	16 (37.20%)	17 (43.60%)	0.915	0.619	0.556
Chorioamnionitis, Number (Percentage)	0 (0%)	1 (2.30%)	0 (0%)	0.293	NC	0.338
PPROM, Number (Percentage)	5 (10.60%)	3 (7%)	4 (10.60%)	0.542	0.954	0.596
Hypothyroidism, Number (Percentage)	0 (0%)	0 (0%)	2 (5.10%)	NC	0.116	0.133
NEC, Number (Percentage)	0 (0%)	1 (2.30%)	0 (0%)	0.293	NC	0.338
Pneumonia, Number (Percentage)	0 (0%)	0 (0%)	1 (2.60%)	NC	0.269	0.291
IVH, Number (Percentage)	0 (0%)	0 (0%)	0 (0%)	NC	NC	NC

a: P values less than 0.05 were considered statistically significant (comparison between groups 1 and 2 using the Chi-Square test).

b: P values less than 0.05 were considered statistically significant (comparison between groups 1 and 3 using the Chi-Square test).

c: P values less than 0.05 were considered statistically significant (comparison between groups 2 and 3 using the Chi-Square test).

* Significance

Abbreviations: NC: Not Calculable PPRM: Preterm Premature Rupture of Membranes NEC: Necrotizing Enterocolitis BPD: Bronchopulmonary Dysplasia IVH: Intraventricular Hemorrhage

Table 3. Comparison of Demographic and Laboratory Data Across the Three Groups

Indicator	Group 1 (Treatment) (n=47)	Group 2 (Treatment + Vitamin D 400 IU) (n=43)	Group 3 (Treatment + Vitamin D 1000 IU) (n=39)	P-value ^a	P-value ^b	P-value ^c
Gravity (Number, Mean ± Standard Deviation)	2.53 ± 1.57	2.44 ± 1.41	2.46 ± 1.51	MW 0.937	MW 0.854	MW 0.924
Maternal Age (Years, Mean ± Standard Deviation)	29.06 ± 6.90	28.13 ± 5.79	29.97 ± 6.12	IS 0.492	IS 0.542	IS 0.167
Gestational Age (Weeks, Mean ± Standard Deviation)	30.89 ± 2.15	30.46 ± 2.13	30.84 ± 1.61	MW 0.376	MW 0.748	MW 0.581
Weight (Grams, Mean ± Standard Deviation)	1602.34 ± 415.90	1580.23 ± 506.76	1618.07 ± 447.47	IS 0.821	IS 0.866	IS 0.722
Apgar 1 (Number, Mean ± Standard Deviation)	6.48 ± 1.06	6.16 ± 1.67	6.33 ± 1.59	MW 0.548	MW 0.971	MW 0.648
Apgar 5 (Number, Mean ± Standard Deviation)	8.08 ± 0.88	7.86 ± 1.33	8.00 ± 1.25	MW 0.782	MW 0.959	MW 0.827
RDS Score (Number, Mean ± Standard Deviation)	6.17 ± 1.30	6.37 ± 1.21	6.30 ± 1.32	MW 0.539	MW 0.672	MW 0.830
Ventilator Time (Mean ± Standard Deviation)	6.80 ± 19.97	3.98 ± 12.92	10.30 ± 31.62	MW 0.308	MW 0.952	MW 0.419
CPAP Time (Hours, Mean ± Standard Deviation)	46.94 ± 41.70	37.98 ± 25.19	36.31 ± 26.29	MW 0.544	MW 0.335	MW 0.717

Hood Time (Mean $\pm$ Standard Deviation)	30.52 $\pm$ 23.24	27.23 $\pm$ 23.88	23.42 $\pm$ 20.66	MW 0.087	*MW 0.008	MW 0.382
Start Feeding Time (Hours, Mean $\pm$ Standard Deviation)	66.75 $\pm$ 48.99	54.86 $\pm$ 28.25	58.92 $\pm$ 40.19	MW 0.513	MW 0.723	MW 0.992
Vitamin D3 1 (ng/dL, Mean $\pm$ Standard Deviation)	18.01 $\pm$ 8.17	15.70 $\pm$ 6.22	18.83 $\pm$ 5.94	IS 0.133	IS 0.595	IS* 0.023
Vitamin D3 2 (ng/dL, Mean $\pm$ Standard Deviation)	30.54 $\pm$ 12.69	47.48 $\pm$ 18.59	49.12 $\pm$ 11.06	*IS 0.001 > P	*IS 0.001 > P	IS 0.633
pH 1 (Number, Mean $\pm$ Standard Deviation)	7.27 $\pm$ 0.11	7.28 $\pm$ 0.09	7.26 $\pm$ 0.08	IS 0.890	IS 0.440	IS 0.331
PCO2 1 (Number, Mean $\pm$ Standard Deviation)	37.57 $\pm$ 16.26	36.48 $\pm$ 14.77	40.25 $\pm$ 15.53	IS 0.740	IS 0.441	IS 0.264
PO2 1 (Number, Mean $\pm$ Standard Deviation)	97.57 $\pm$ 45.13	10571 $\pm$ 40.01	10013 $\pm$ 48.89	MW 0.542	MW 0.655	MW 0.933
HCO3 1 (Number, Mean $\pm$ Standard Deviation)	16.99 $\pm$ 2.73	16.94 $\pm$ 2.71	16.97 $\pm$ 13.19	IS 0.932	IS 0.977	IS 0.963
Base Excess 1 (Number, Mean $\pm$ Standard Deviation)	11.58- $\pm$ 3.21	11.74- $\pm$ 2.54	11.01- $\pm$ 4.54	MW 0.907	MW 0.872	MW 0.867
Total Calcium (Number, Mean $\pm$ Standard Deviation)	9.16 $\pm$ 0.75	8.93 $\pm$ 0.78	8.73 $\pm$ 0.86	MW 0.154	*MW 0.010	MW 0.259

Ionized Calcium (Number, Mean $\pm$ Standard Deviation)	1.17 $\pm$ 0.12	1.33 $\pm$ 1.36	1.12 $\pm$ 0.11	MW 0.139	MW 0.102	MW 0.791
Admission Time (Days, Mean $\pm$ Standard Deviation)	18.51 $\pm$ 12.15	18.62 $\pm$ 15.29	15.07 $\pm$ 8.84	MW 0.660	MW 0.270	MW 0.542

a: P values less than 0.05 were considered statistically significant (comparison of mean and standard deviation between groups 1 and 2 using the t-test).

b: P values less than 0.05 were considered statistically significant (comparison of mean and standard deviation between groups 1 and 3 using the t-test).

c: P values less than 0.05 were considered statistically significant (comparison of mean and standard deviation between groups 2 and 3 using the t-test).

IS: Due to the normality of the data, confirmed by the Shapiro-Wilk and Kolmogorov-Smirnov tests, the Independent Samples Test was used.

MW: Due to the non-normality of the data, confirmed by the Shapiro-Wilk and Kolmogorov-Smirnov tests, the Mann-Whitney U test was used.

* Significance

Table 4. Comparison of Total Oxygen Therapy Hours Across the Three Groups

Indicator	Group 1 (Treatment) (n=47)	Group 2 (Treatment + Vitamin D 400 IU) (n=43)	Group 3 (Treatment + Vitamin D 1000 IU) (n=39)	P-value ^a (Kruskal-Wallis)
Total Oxygen Therapy Hours (Hours, Mean $\pm$ Standard Deviation)	70.53 $\pm$ 25.60	63.66 $\pm$ 22.30	20.80 $\pm$ 59.76	0.393

a: The non-parametric Kruskal-Wallis test was used because the distribution of the total oxygen therapy hours variable was not normal, as confirmed by the Shapiro-Wilk and Kolmogorov-Smirnov tests.

Table 5. Comparison of Time to full feeds Across the Three Groups

Indicator	Group 1 (Treatment) (n=47)	Group 2 (Treatment + Vitamin D 400 IU) (n=43)	Group 3 (Treatment + Vitamin D 1000 IU) (n=39)	P-value ^a (Kruskal- Wallis)
Time to full feeds (day, Mean ± Standard Deviation)	151.02 ± 14.39	124.06 ± 8.39	128.97 ± 12.68	0.686

a: The non-parametric Kruskal-Wallis test was used because the distribution of the total oxygen therapy hours variable was not normal, as confirmed by the Shapiro-Wilk and Kolmogorov-Smirnov tests.

Table 6. Comparison of Vitamin D Levels, Ionized Calcium, and Respiratory Score

Variables	Spearman Correlation Coefficient (ρ)	P-value
Group 1 (Vit D3 1) and RDS Score	-0.105	0.235
Group 2 (Vit D3 2) and RDS Score	0.049	0.580
Ionized Calcium (Ca I) and RDS Score	0.228	*0.009

* Significance

Table 7. Effect Size (Odds Ratio) for RDS Improvement Outcome in the Three Groups

Compared Groups	Odds Ratio (Exp(B))	P-value
Group 1 vs. Group 2 (400 IU)	-17.37	0.99
Group 1 vs. Group 3 (1000 IU)	-17.37	0.99

Table 8. Association Between Vitamin D Levels and RDS Score Between Group 4 and Groups 1 to 3 (Group 5)

Compared Group	RDS_Improved Status	Odds Ratio (Exp(B))	P-value
Group 4 (Normal Vitamin D)	Improved = 1	Baseline	-
Group 5 (Groups 1-3)	Improved = 1	0.33	0.44
	Not Improved = 0	3.00	0.44

Figures

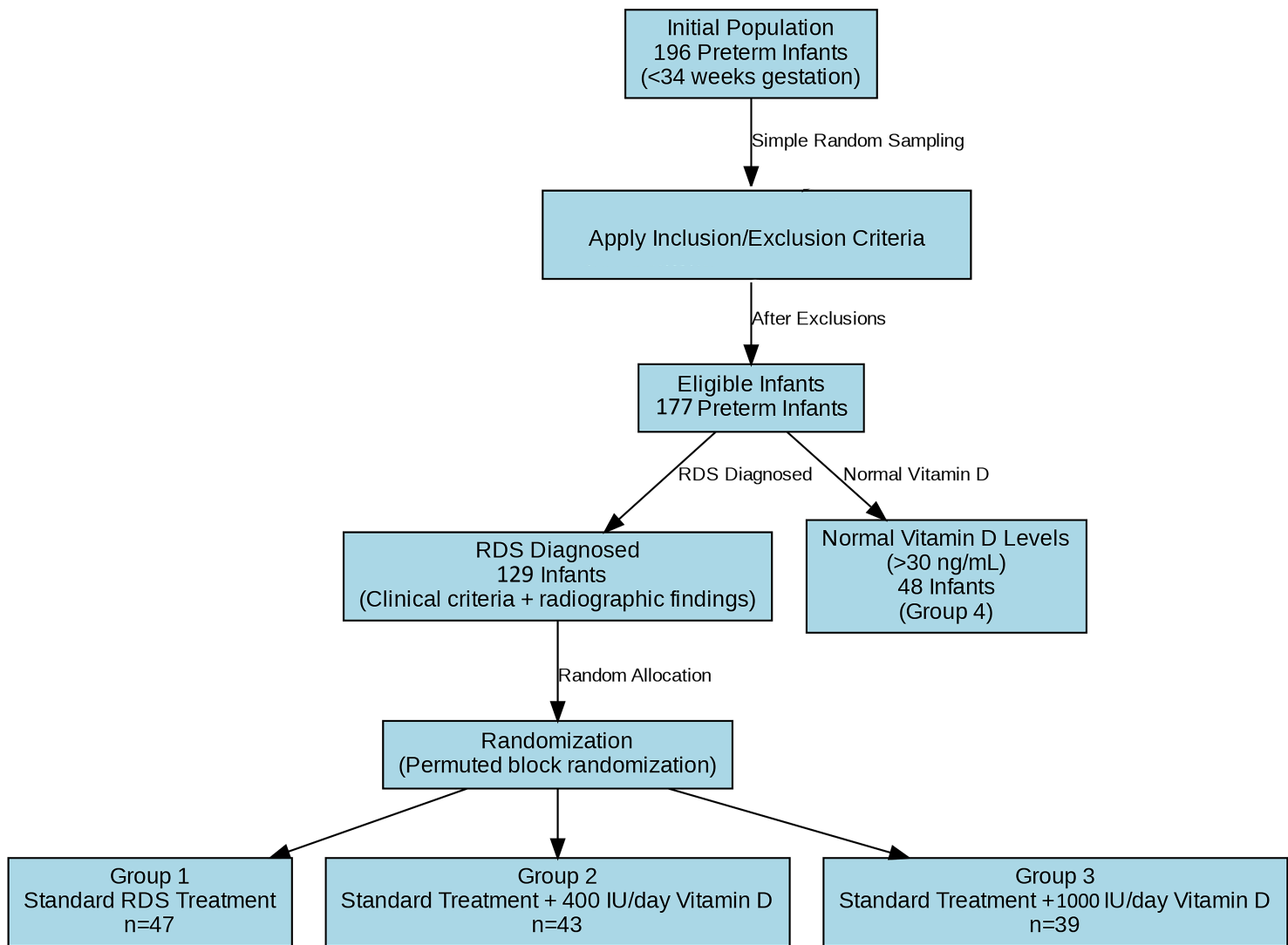


Figure 1

The flow diagram of the sampling process.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [CONSORT2025editablechecklist.docx](#)