# A New Approach to Prevent Neonatal Respiratory Distress Syndrome: A randomized clinical trial of 25(OH) D Injections before Preterm Delivery

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**Research Article**

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# Abstract

Background

Vitamin D deficiencies have been suggested as one of the risk factors for neonatal respiratory distress syndrome (RDS). The present study aimed to evaluate the effect of **25-hydroxyvitamin D [25(OH)D]** administration in pregnant women at risk of preterm delivery on the incidence of RDS in their preterm neonates.

Methods

A single-blinded randomized controlled clinical trial was conducted on pregnant mothers with a gestational age of less than 34 weeks at risk of preterm delivery. Subjects were randomly assigned into two groups by the corresponding author, including intervention (intramuscular injection of 50,000 units of 25(OH) D three days before delivery) and control (with no injection of 25(OH) D). Serum concentrations of 25(OH) D were measured after offspring, including RDS, were collected and recorded in a checklist. Short-term outcomes and the need for respiratory support were assessed by the principal investigator who was unaware of the type of intervention. Data were analyzed by independent t-test, paired sample t-test, and chi-square test.

Results

Despite the homogeneity of neonates in the two groups in terms of gestational age, birth weight and the delivery method, 45% of neonates in the control group and 20% in the intervention group developed respiratory distress syndrome ($P < 0.05$). The mean 25(OH) D level in neonates was 17.7 ± 10.5 and 23.7 ± 13.5 ng/mL in the intervention and control groups, respectively.

Conclusions

According to this study, a single dose of 50,000 units of intramuscular 25(OH) D in pregnant women at risk of preterm delivery can reduce the incidence of RDS in the newborn.

Trial Registration

Iranian Registry of Clinical Trials, IRCT20190814044529N1. Registered 21st Jan. 2020, https://fa.irct.ir/user/trial/41515/view


# Background

Neonatal Respiratory Distress Syndrome (RDS) is a significant cause of mortality and morbidity in preterm neonates besides leading to a remarkable rise in NICU-hospitalization related costs. The incidence of RDS is inversely proportional to the neonate's weight and gestational age; the highest incidence being in the 22nd to 24th week of gestation which reduces to 25% at the neonatal weight of

1251 to 1500 grams. Many interventions have been performed in pregnant women at risk of preterm delivery, hoping to reach greater maturity of the fetal lungs, especially regarding the lungs development and adequate surfactant function. The only intervention with beneficial results has been prenatal corticosteroids. Antenatal corticosteroids cause structural maturation of the fetal lungs, which of course, is not associated with an increased volume of alveolar surfactant (1).

Recently, much attention has been paid to maternal 25(OH) D deficiency during pregnancy and its detrimental outcomes for mothers and neonates, especially among pregnant women at risk of preterm delivery; although this claim is still not widely accepted. This clinical trial aimed to evaluate the consequences of a single-course of antenatal intramuscular injection of 25(OH) D for pregnant women at risk of preterm delivery on the incidence and severity of neonatal respiratory distress syndrome.

## Methods

# Study design

This single-blind randomized controlled trial, using the randomization box, was conducted in the Department of Obstetrics and Gynecology and the Intensive Care Unit of Qaem Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran, between January 21st, 2019 and August 23rd 2019. An informed consent was obtained from each participant by a single researcher prior to study entrance. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.fm.REC.1395.52) and was registered in the Iranian Registry of Clinical Trials (IRCT20190814044529N1; 21/01/2020).

All singleton pregnant women with a gestational age of less than 34 weeks and at risk of preterm delivery, normal prenatal ultrasound screening results, and no significant medical and obstetrical conditions regarding the mother (such as cardiovascular disorders, diabetes mellitus, epilepsy, and asthma) were included in the study.

The exclusion criteria was as follows: a gestational age of ≥ 34 weeks, neonatal death at birth, major congenital anomalies, severe perinatal asphyxia, necrotizing enterocolitis, stillbirth, respiratory distress except for neonatal respiratory distress syndrome, and neonatal abstinence syndrome.

# Randomization

Pregnant mothers at risk of preterm labor were assigned to the intervention and control arms of the study based on simple randomization with respect to the inclusion criteria. Initially, 50,000 units of 25(OH) D was injected intramuscularly for mothers in the intervention group. The principal researcher evaluated the neonates' outcome including respiratory distress, unaware of group assignments. The collected data were classified as groups A and B in the SPSS software, and a statistical analyst who was blind to group assignments interpreted the data. Due to the lack of similar studies in the literature, this study is regarded

as a pilot study. A sample consisting of at least 20 cases and 20 controls was considered to fulfill the comparisons. Therefore, in total 175 mothers were enrolled in the study.

# Data collection

Medical data relevant to the study were documented in the patients' medical records. Maternal data included age, parity, antenatal corticosteroids and vitamin D supplements consumption, and gestational age at the time of admittance and delivery. The neonatal data consisted of gestational age, gender, birth weight, Apgar scores at 1 and 5 minutes, hospitalization in the neonatal intensive care unit, the need for ventilation support, surfactant replacement therapy for RDS, length of hospital stay, morbidities and mortalities due to prematurity such as necrotizing enterocolitis and infection (based on positive blood culture).

# Study intervention

Pregnant women in the intervention group who had not taken any 25(OH) D supplements during pregnancy were injected with a single dose of 50,000 units of intramuscular 25(OH)D within 72 hours of preterm delivery.

# Laboratory evaluation

A 1.5 ml blood sample was taken from both neonates and mothers after delivery to check the vitamin D level. The prepared samples were kept at -20°C and sent to the central laboratory of Qaem Hospital for analyses. Vitamin D level was measured in ng/mL by the ELISA method using an Elisa Reader (RT2100c, Germany) and Elisa Washing devices. A 25(OH) D level of less than 30 ng/mL was considered as vitamin D deficiency and a level ≥ 30 ng/mL was considered as the sufficient value. Subjects with vit D deficiency were divided into three groups according to their serum 25(OH) D level: severe deficiency (≤ 10 ng/mL), moderate deficiency (10.1–20 ng/mL), and mild deficiency (20.1–30 ng/mL). Other related laboratory studies based on the local neonatal guidelines including the quantitative assessment of CRP, CBC, and blood culture were also performed.

# Radiological evaluation

All hospitalized neonates with respiratory problems underwent chest x-rays. The radiographic findings used for confirming the diagnosis of neonatal respiratory distress syndrome included bilateral and relatively symmetric diffused ground glass opacities with low volumes and a bell-shaped thorax, air bronchograms, and absence of hyperinflation.

The criteria for diagnosing respiratory distress syndrome were: respiratory distress findings including tachypnea (> 60 /min), intercostal muscle retraction, grunting and nasal flaring; chest radiographic findings as mentioned previously, the need for respiratory support under nasal CPAP with a PEEP > 8 cmH2O and/or FiO2 > 30, and abnormal arterial blood gas analysis.

# Patients and Methods

This study was a clinical trial conducted on 175 pregnant women with a GA of less than 34 weeks at birth. Among them, 25 cases from the intervention group were placed under nasal continuous positive airway pressure (NCPAP) therapy with FiO2 of 30% and PEEP of 5 cmH2O at the time of admission. Neonates in whom respiratory support, including increasing PEEP to > 8 cmH2o with Fio2 > 30, surfactant administration and extubation and or endotracheal intubation had been introduced met the inclusion criteria. All subjects were monitored continuously by routine observation and pulse oximetry. Neonatal variables including blood gas analysis (pH, PO2, PCO2), oxygen saturation, gender of the infant, birth weight and gestational age were recorded. The neonatal primary outcome measure in the study group was respiratory distress syndrome and its incidence in neonates born less than 34 weeks of gestation. Diagnosis of sepsis was based on the observation of clinical signs and positive blood culture. Diagnosis of necrotizing enterocolitis was based on the Bell staging criteria, including systemic symptoms severity and radiographic findings.

# Statistical analysis

Statistical analysis was performed by the SPSS software (IBM SPSS Statistics, Version 21). Independent t-test or Mann-Whitney test were used to compare quantitative variables between the two groups. Quantitative variables within each group were compared with paired sample t-test. To compare qualitative variables between the two studied groups, Chi-square test and Fisher's exact tests were used. Data are presented as mean ± standard deviation (SD). A P-value < 0.05 was considered as statistically significant.

# Results

One hundred seventy-five pregnant women with a gestational age of less than 34 weeks, including 88 cases and 87 controls, were included in the study. In total, 89 subjects were excluded due to stillbirth, major anomalies, severe asphyxia, and gestational age at or over 34 weeks at birth. Among the 86 remaining cases, 51 women who did not receive 25(OH)D were assigned to the control group, and 35 others who received a single dose of intramuscular 25(OH)D were placed in the intervention group. Ten neonates in the intervention group were excluded due to neonatal abstinence syndrome, sepsis, necrotizing enterocolitis, and drop out from the study by the parents. Moreover, 11 neonates in the control group were excluded due to neonatal abstinence syndrome, sepsis, transient neonatal tachypnea, and drop out from the study by the parents. Subsequently, the statistical analysis was performed on 65 neonates, including 25 cases and 40 controls (Figure 1).

In this study, no significant difference was achieved in the mean birth weight, gestational age, delivery method, hospitalization in the intensive care unit, length of hospital stay, gender, 1st and 5th minute Apgar scores, and time to death between the two groups (P> 0.05; Table 1). Sixty-four percent of neonates in the intervention group and 52.5% in the control group were male. The delivery method was cesarean section in 92% and 75% of the cases and controls, respectively. The mean 25(OH)D level in mothers of the intervention and control groups was 24.6 ± 9.3 and 23.3 ± 14.6 ng/mL, respectively. The incidence of 25(OH)D deficiency (<30 ng/mL) in the neonates of the aforementioned groups was 96% and 74.3%,

respectively (Figure 2). The 25(OH)D level was normal in 4% and 25.7% of neonates, and 28% and 14.30% of mothers in the intervention and control groups, respectively (Figure 2). Twenty-three neonates, including 5 cases and 18 controls required surfactant replacement therapy (Table 1). The highest incidence of RDS in both the intervention and control groups was in the 25(OH)D deficiency subgroup. In the neonates of the intervention group, no case of RDS were observed in the subgroup with normal vitamin D levels; whereas in the control group, 9.5% of the cases in the mentioned subgroup and 14.3% of those with severe vitamin D deficiency suffered from RDS (Figure 3).

Table 1: Clinical and laboratory characteristics of premature neonates in the intervention and control groups. Data are expressed as mean ± SD

| Parameter | Intervention (n=25) | Control (n=40) | P-value | |
|---|---|---|---|---|
| Maternal 25(OH)D level | 24.6±9.3 ng/mL | 23.3±14.63 ng/mL | 0.215 | * |
| Neonatal 25(OH)D level | 17.7±10.53 ng/mL | 23.7±13.53 ng/mL | 0.074 | * |
| 1st APGAR score | 7.00±2.3 | 6.7±2.1 | 0.283 | * |
| 5thmin APGAR score | 9.00±0.9 | 8.2±1.2 | 0.003 | * |
| Gestational age (wks.) | 32.5±1.44 | 31.8±1.98 | 0.186 | * |
| Delivery by cesarean section | 23(92%) | 30(75%) | 0.109 | ** |
| Birth weight (gr) | 1767±358.9 | 1621.7±411.4 | 0.150 | **** |
| Sex (male) | 16(64%) | 21(52.5%) | 0.362 | *** |
| Hospital admission | 19(76%) | 35(87.5%) | 0.097 | ** |
| Days of hospitalization | 13.7±9.4 | 20.3±17.3 | 0.250 | * |
| Death | 3(12%) | 5(12.5%) | 0.097 | ** |
| Time from birth to death (day) | 10(10-30) | 6(5-48) | 0.114 | * |
| RDS cases treated by surfactant | 5(20%) | 18(45%) | 0.04 | *** |

* Mann–Whitney U test

** Fisher's exact test

*** Chi-square test

**** Independent t-test

# Discussion

According to our findings, despite the similarity of the two groups in terms of gestational age, birth weight and type of delivery, administration of a single course of intramuscular vitamin D injection to pregnant

women less than 34 weeks of gestation reduced the incidence of respiratory distress syndrome by less than half (from 20–45%). To the best of our knowledge, this is the first clinical trial performed in the human population on pregnant mothers with preterm delivery symptoms evaluating the effect of vitamin D on the incidence and severity of RDS.

A systematic review on the level of 25(OH) D in pregnant women and neonates worldwide found that vitamin D deficiency [25(OH)D < 50 nmol/L (20 ng/mL)] is more prevalent in pregnant women and newborns than described previously (2). It has been shown that vitamin D has significant effects on the prenatal and postnatal growth and development of the lungs in animal models and human beings (3, 4). The role of 25(OH)D in fetal lung maturation, lung volume, the biosynthesis of surfactant phospholipids (SP-B), stimulating surfactant release, and the maturation of type II pneumocytes has been confirmed in animal models (5); it is also involved in alveolar mesenchymal-epithelial reactions (6). On the one hand, some studies have shown that vitamin D deficiency can lead to lower oxygen saturation and shorter survival time in premature animals, perhaps due to low fetal lung weight resulting from lack of proper development (7).On the other hand, lung molecular and functional abnormalities in animal models can be prevented by vitamin D supplementation (8). Even though the effects of the antenatal administration of 25(OH)D on the incidence of respiratory distress syndrome in premature human neonates have not been studied, vitamin D deficiency is a frequent finding in human preterm neonates suffering from RDS (9, 10). Most studies in this field have been focused on the correlation between maternal and neonatal 25(OH)D levels (11–13) regardless of the neonatal outcomes. Based on animal studies, many researchers believe that 25(OH)D prevents respiratory distress syndrome by increasing the proliferation of type 2 pneumocytes. Recent studies have demonstrated an association between 25(OH)D deficiency and an increase in respiratory distress syndrome. Boskabadi et al. (14) have shown a positive relationship between 25(OH)D deficiency and an increase in the rate of respiratory distress syndrome (9). Hegazy et al. (15) also showed that neonates with respiratory distress syndrome had lower serum 25(OH)D levels than controls. Dogan et al. (16) found that 68% of neonates with respiratory distress syndrome have vitamin D deficiency; Fettah et al. (17) concluded that the risk of respiratory distress syndrome was very high in vitamin D deficient neonates.

Our study proved that increasing 25(OH) D levels in both mothers and neonates is a significant factor in reducing the neonates' respiratory distress syndrome incidence and severity. The higher the level of 25(OH) D, the lower the incidence and the severity of respiratory distress syndrome. The most apparent difference between the neonates in the intervention and control groups was the absence of respiratory distress syndrome in a subgroup of the study group in which 25 (OH) D levels was normal or severely deficient. The absence of RDS in the subgroup with severe 25(OH)D deficiency may be due to the direct effect of 25(OH)D on target tissues, including type 2 pneumocytes, before altering serum levels of 25(OH)D.

The main limitation of the present study was the uncertainty of the cause of preterm delivery and the lack of maternal 25(OH) D level measurement before injection. We recommend a higher dose of 25(OH) D than that of this trial for future studies. Moreover, before examining the relationship between neonatal

25(OH) D level and the incidence of respiratory distress syndrome, the serum level of maternal 25(OH) D should be tested both before and after vitamin D supplementation and with the aim of reaching the normal level following administration.

# Conclusions

According to our study results, the injection of 50,000 units of intramuscular 25(OH) D within 72 hours before delivery can reduce the RDS incidence by half, and this effect has been more prominent in severe cases of 25(OH)D deficiency. Therefore, 25(OH) D along with corticosteroids may improve the prognosis of preterm neonates.

# Abbreviations

**RDS**
respiratory distress syndrome
**25(OH) D**
25-hydroxyvitamin D
**FIO2**
fraction of inspired oxygen
**PEEP**
positive end-expiratory pressure
**NCPAP**
nasal continuous positive airway pressure

# Declarations

**Ethics approval and consent to participate:** The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.fm.REC.1395.52). An informed consent was obtained from each participant by a single researcher prior to study entrance. All methods were performed in accordance with the relevant guidelines and regulations

**Consent for publication:** Not applicable

**Availability of data and materials:** The datasets used during the current study are available from the corresponding author on reasonable request. Identifying/confidential patient data could not be shared.

**Competing interests:** The authors declare that they have no competing interests

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**Authors' contributions:** HB and GM have made substantial contributions to the conception and design of the work, GM and NS had a significant role in data acquisition and interpretation, MHAT had a major role in data acquisition and drafted the work. All authors have read and approved the submitted version of the manuscript."

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# Figures

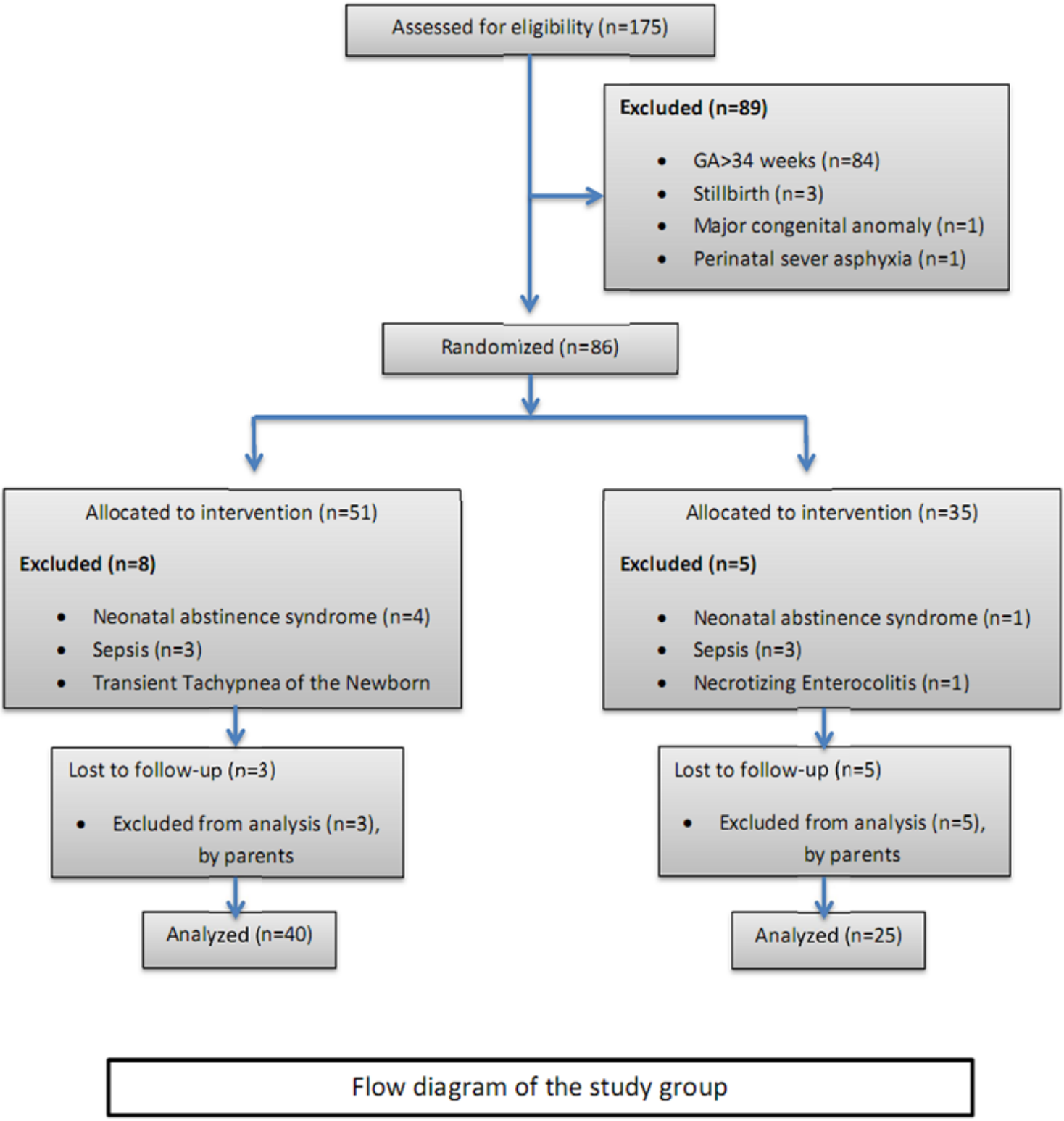


**Figure 1**

Consort Flow Diagram of the studied participants

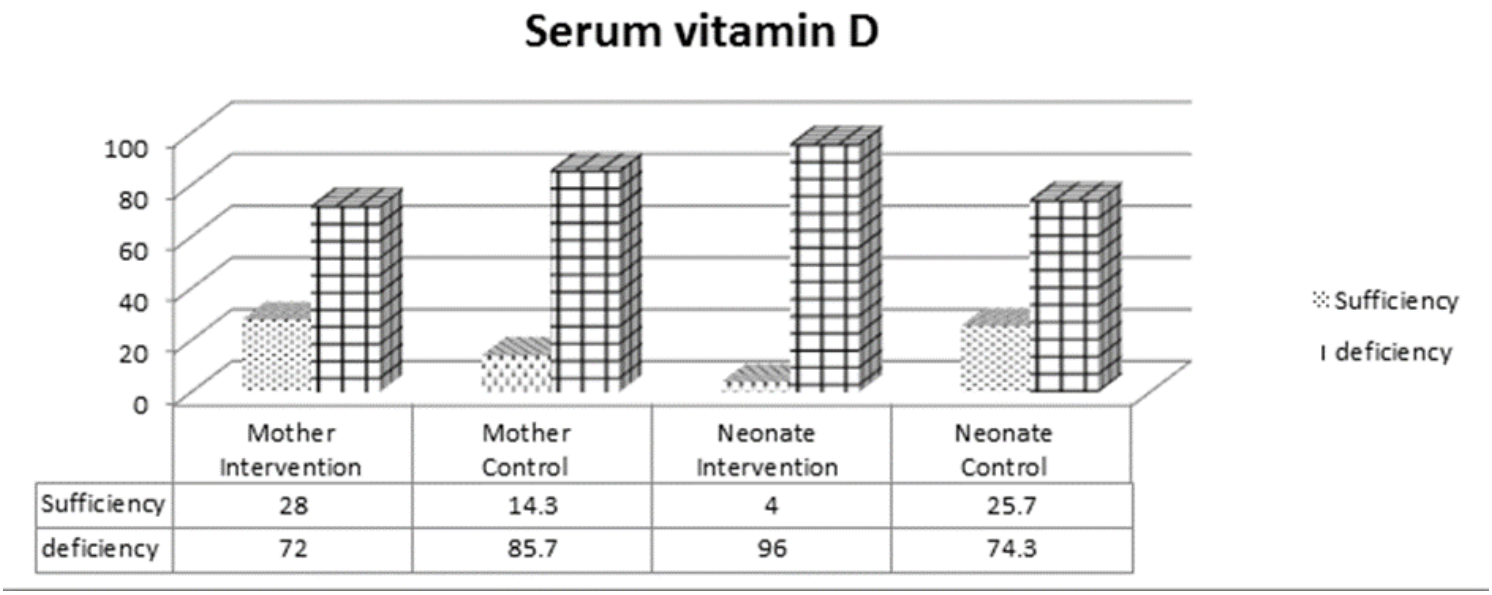


**Figure 2**

Percentage of vitamin D level in the mothers and neonates of the two studied groups

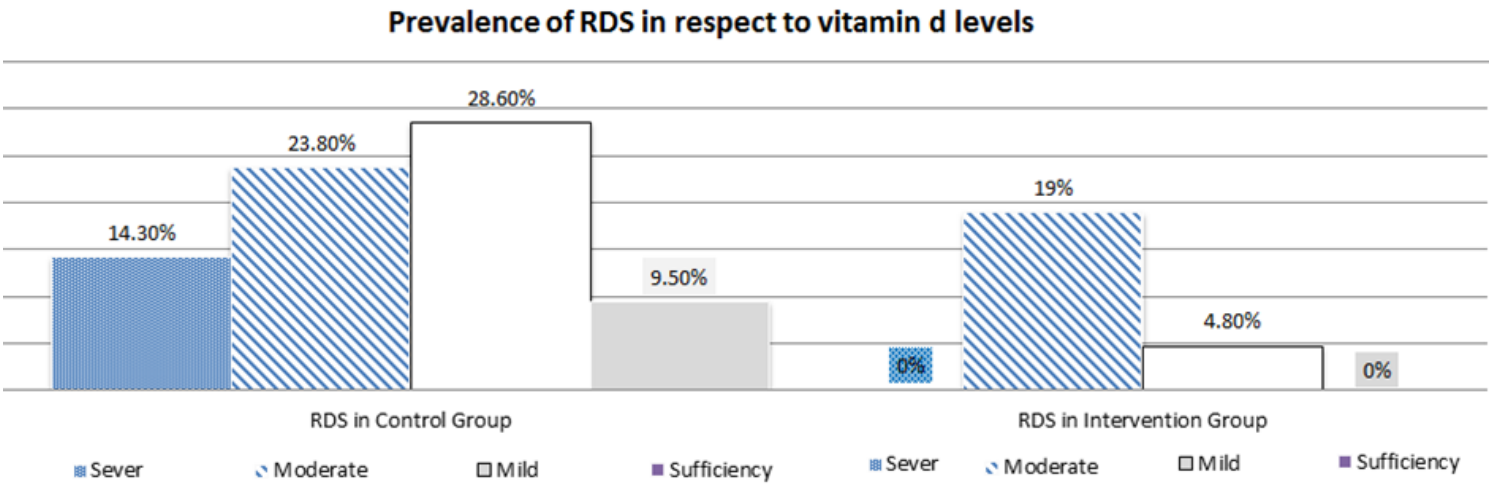


**Figure 3**

RDS percentage in the intervention and control groups, in relation to 25(OH)D levels

# Supplementary Files

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- CONSORT2010checklistfirst.doc
- finalProjectsummary.docx