

## Effect of Vitamin D Supplementation on Vitamin D Levels of Term and Preterm Neonates and their Mothers: A Clinical Trial Study

\*Bahareh Tanbakuchi<sup>1</sup>, Fatemeh Taheri<sup>2</sup>, Mahmoud Zardast<sup>3</sup>, Abbasali Ramazani<sup>4</sup>

<sup>1</sup>Resident of Pediatrics, Department of Pediatrics, Faculty of Medicine, Birjand University of Medical Sciences (BUMS), Birjand, South Khorasan Province, Iran. <sup>2</sup>Professor, Atherosclerosis and Coronary Artery Research Center, Department of Pediatrics, Faculty of Medicine, Birjand University of Medical Sciences (BUMS), Birjand, South Khorasan Province, Iran. <sup>3</sup>Associate Professor, Atherosclerosis and Coronary Artery Research Center, Department of Pathology, Faculty of Medicine, Birjand University of Medical Sciences (BUMS), Birjand, South Khorasan Province, Iran. Social Determinants of Health Research Center, Department of Epidemiology and Biostatistics, Birjand University of Medical Sciences, Birjand, Iran.

### Abstract

**Background:** Vitamin D deficiency is a worldwide issue and the effect of vitamin D supplementation alone as a possible option to prevent that is still under examination. Therefore, the aim of this study was to evaluate the effect of vitamin D supplementation on term and preterm neonates and mothers for 4 months.

### Materials and Methods

In this clinical trial, 30 term neonates and 30 preterm neonates along with their mothers created a total of 120 participants who were referred to the maternity unit of Vali-e-Asr hospital in Birjand, Iran, in 2018. Neonates and mothers were supplemented with vitamin D supplementation of 400 IU/day and 50,000 IU/month of vitamin D drops and tablets, respectively. Serum vitamin D levels of mothers and infants were measured during delivery, birth and after 4 months using ELISA method.  $P < 0.05$  was considered as a significant level.

**Results:** The level of vitamin D in term and preterm neonates was  $24.53 \pm 10.93$  and  $41.30 \pm 12.87$ ,  $19.45 \pm 8.88$  and  $43.78 \pm 15.51$  ng/ml at birth and at 4 months of age, respectively. There was a significant difference between the mean level of vitamin D at birth and 4 months later in both infants and mothers ( $P < 0.001$ ). There was also a significant positive correlation between vitamin D levels of mothers during labor and neonates at birth ( $P < 0.001$ ).

### Conclusion

Although vitamin D deficiency was high, regular consumption of vitamin D supplements has a significant effect on vitamin D levels and could increase the level of vitamin D to the normal levels, particularly in neonates.

**Key Words:** Mothers, Neonate, Supplementation, Vitamin D.

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### \* Corresponding Author:

Bahareh Tanbakuchi (M.D), Address: Department of Pediatrics, Faculty of Medicine, Birjand University of Medical Sciences (BUMS), South Khorassan, Birjand, Iran. Postal code: 971796151.

Email: baharehtanbakuchi@yahoo.com

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## 1- INTRODUCTION

Vitamin D plays an important role in bone evolution and muscle function. It facilitates the absorption of calcium and phosphorus from the kidneys and intestines, it can inhibit parathyroid hormone, and is essential for bone formation and development (1). 90% of vitamin D in the skin is made of sunlight and the rest (10%) is nutritional (2). Therefore, the use of foods contains vitamin D and exposure to direct sunlight is essential for daily intake of this vitamin. The World Health Organization/United Nations Food and Agriculture Organization (FAO/WHO) recommended a diet of 200 IU/day vitamin D for pregnant women. Dietary sources of vitamin D include food and dietary supplements (3, 4).

Since vitamin D plays a major role in regulating blood calcium and hemostasis, studies have shown that deficiency of this vitamin can contribute to deterioration of certain diseases such as arterial blood pressure, diabetes, and heart failure (5). Blood levels examination of vitamin D (25-hydroxyvitamin D: 25(OH)D) is a good way to estimate the nutritional status of vitamin D. Recently, an optimal level of 25 hydroxyvitamin D has been reported by some scientists at 20-30 ng/ml and a lower level of 10 ng/ml is a severe deficiency of this vitamin (6). Vitamin D deficiency in pregnancy is associated with increased risk of preeclampsia, diabetes mellitus, preterm delivery, small infant size relative to the gestational age, impaired fetal skeletal development causing rickets in the infant, bone loss and other specific tissue problems (7, 8). Despite the progress made in the prevention and treatment of nutritional rickets, it is still shown to be strongly associated with vitamin D deficiency and therefore presenting as a major health problem in many developing countries. Accordingly, reports of such prevalence in developed countries are also

rising (9, 10). Various studies carried out over the past two decades have reported a high prevalence of vitamin D deficiency in a range of 30 to 93% in countries such as China, Turkey, India, Saudi Arabia and Iran (11). Hollis and Wagner (12) found that 12% of American women aged 20-29 years old (peak years of reproduction) had a lower serum vitamin D level than was expected for vitamin D deficiency. Deficiency of vitamin D in pregnant mothers reduces the transfer of vitamin D through placenta and the birth of embryos at birth (13). The 25-OH-D blood level in breast-fed infants is directly related to the amount of vitamin D in breast milk (14).

Lactating mothers are often vitamin D deficient, leading to reduced calcium concentration of their milk and ultimately creating vitamin D deficiency in their infants (15). For this reason, in early infancy, the level of vitamin D of infants who are exclusively or predominantly breast-fed is dependent on the amount of vitamin D received through placenta in embryonic life. Therefore, the status of maternal vitamin D in preventing vitamin D deficiency in infants is very important (15). So, adequate concentration of vitamin D during pregnancy is needed for proper maternal responses to the need for calcium in the fetus and calcium usage by infants (16). Given the available evidence, it is better to implement vitamin D intake as part of pre-natal care routine in order to improve maternal and neonatal outcomes for all mothers. While some evidence suggests that giving vitamin D supplementation can reduce the risk of high blood pressure and increase the height and size of infant's head at birth, to confirm these effects, more accurate randomized trials are required (17). Currently, the number of high-quality trials and large sample sizes reported are very limited for arriving at a scientific conclusion about the usefulness and safety of vitamin D supplementation (17).

Regarding the high prevalence of vitamin D deficiency and the different results obtained from available studies in this regard and considering the implementation of the national plan for lactating mothers and infants, no program has been developed since 2016 to evaluate the precise impact of vitamin D supplementation in mothers and infants (term and preterm). Therefore, the aim of this study was to evaluate the effect of vitamin d supplementation on vitamin D levels of term and preterm neonates and their mothers who referred to Vali-e-Asr Hospital of Birjand, Iran.

## **2- MATERIALS AND METHODS**

### **2-1. Study design and population**

In this clinical trial, using a non-probability sampling technique, 30 breastfeeding mothers with their 30 term infants, and 30 mothers with their 30 preterm newborns (total of 120 mothers and neonates), were selected and studied among the newborns and their mothers referring to the maternity unit of Vali-e-Asr Hospital in Birjand, Iran, in 2018.

### **2-2. Methods**

First, the purpose of the research project was explained to the mother, and if they wished and signed the consent form, their evaluation started. Neonates were provided with standard vitamin D drops supplementation of 400 units/day and mother with vitamin D tablets of 50,000 units/month (3, 16). After infants' 4th month of age, the vitamin D serum level was evaluated. The maternal and cord blood levels of the term and preterm infants were measured at the time of delivery. Serum samples were collected and after sampling was done, the experiments were tested one at a time using a vitamin D kit. Every month in the follow-up stage, mothers were referred to the pediatrics unit of the hospital and monitored for breastfeeding as well as

supplementing the infants with one drop of vitamin D. Samples lacking vitamin D or under exclusive breastfeeding were excluded from the study. Mothers and infants at 4 months of age were evaluated for serum vitamin D levels. Serum levels below 30 ng /mL were labeled as vitamin D deficiency (6, 18). Sampling (5 ml) of term and preterm infants was performed by the neonate's midwife in the first stage from the umbilical cord. The mothers sampling (5 ml), also was conducted after about four hours of giving birth. After four-months, blood samples were taken from the back of the infant's hand and mother's cubital vein by the pediatric resident, transferred to the BD American gel tubes (3 ml) containing clot activator and centrifuged for an hour and the serum was used for examination. The results of the experiments were calculated in ng/ml.

### **2-3. Laboratory measurements**

The demographic form was designed by the supervisory team at the study design stage and completed by the pediatric resident through examining and interviewing the mother. The form included demographic information (e.g. age, weight, height, body mass index, educational level, employment, place of residence, number of children, number of births, gestational age, breastfeeding history in previous children, specific illness or treatment, and drug and/or supplement intake). The subjects completed physical examination done by podiatrist and pediatric resident. All neonate heights were examined with an infant meter (made in Iran, with an error of 0.5 cm) at the time of birth. Weight measures and head circumference were taken and obtained data were recorded by pediatric resident. Weighing of the neonates was done by digital scale (German Seca Model) with 100 g calibration weight. Neonatal length was measured by pediatric resident and by a length-board. Subsequently, body mass

index (BMI) of the mothers was measured at the time of labor. The serum samples were isolated after centrifugation and kept at  $-20^{\circ}\text{C}$  until the laboratory assessments. The serum 25(OH) D levels were measured using the ELISA method (Euroimmun kit, Medizinische Labordiagnostika AG, Germany). The 25-OH Vitamin D ELISA is designed for serological determination of vitamin D concentrations in humans. The serum level of 25-OH vitamin D, which stores the most metabolites in the blood, is a suitable indicator of the actual vitamin D supply in the human body. 25-OH vitamin D from the patient sample is bound by anti-25-OH antibodies on the microplate. Free antibody binding sites are occupied by labelled 25-OH vitamin D. The intensity of the color formed after adding the chromogen/substrate solution is measured using a photometer. The color intensity is inversely proportional to the 25-OH vitamin D concentration in the serum or plasma. Tests were performed two times and the averaged values were used in the analyses to increase the accuracy of the results. The following formula can be used to convert a unit of vitamin D:  $\text{ng/ml} \times 2.5 = \text{nmol/L}$ . According to the guidelines of the American Endocrine Association, vitamin D level is defined with the concentration of 25-hydroxyvitamin D<sub>3</sub> in blood. Deficient, insufficient, and natural vitamin D levels were determined with 25-hydroxyvitamin D level lower than 20 ng/ml, 21–29 ng/ml, and 30 ng/ml, respectively (18).

#### **2-4. Ethical consideration**

Ethical considerations in this study are specified as following:

- Ethics approval was obtained from the Ethics Committee of the Birjand University of Medical Sciences (approved by the Ethics Committee: IR.BUMS.REC.1397.116).

- Full parental consent forms were signed by the parents.
- No charge was included for the infant's families.
- Infants were referred to a specialist for follow-up if diagnosed with any disease or disorder.
- This project was registered in the Thai Registry of Clinical Trials system and has a clinical practice code of TCTR20190808002.

#### **2-5. Inclusion and exclusion criteria**

Mothers and infants were entered to the study with no previous known disease (i.e. heart disease, kidney disease etc.), and no pregnancy complications such as deformity, preeclampsia, etc. as well as willingness to participate in the study. As another inclusion criteria of this study, the mothers who have only received vitamin D supplementation during pregnancy and resided in Birjand were examined. Neonates and their mothers were excluded if they did not meet the selection criteria above.

#### **2-7. Data Analysis**

Data were analyzed using SPSS software version 22.0 (IBM Corp. IBM SPSS Statistics for Windows). Mean, standard deviation (SD), and frequency distribution were separately used to describe the research sample. Next, quantitative variables of the normal distribution were determined by the Kolmogorov-Smirnov test. Descriptive and analytical statistics were used and analyzed by Chi-square test, Pearson correlation coefficient, paired t-test and independent t-test.  $P < 0.05$  was considered as a significant level.

### **3- RESULTS**

In this study, 30 mothers with their 30 term neonates along with 30 mothers and 30 preterm neonates (total of 120 individuals) were studied. In the second stage of the study (4 months after birth),

from the 30 neonates in the first stage, 8 infants in two groups (4 in each group) were removed from participation in this study (because of reluctance to continue to study), and each group was performed with 26 newborns and mothers in the second stage. The mean age of mothers of term and preterm neonates during labor was  $28.43 \pm 5.48$  years and  $29.67 \pm 5.62$  years old, respectively. The mean BMI of term and preterm infants' mothers was  $28.91 \pm 5.02$  and  $28.28 \pm 5.51$ , respectively. As shown in **Table.1**, 13 and 18, 17 and 12 of the term and preterm infants were male and female, respectively, and there was no significant difference in gender between the two groups. The mean height and weight of term and preterm newborns were

$50.55 \pm 2.32$  and  $63 \pm 2.21$  cm,  $46.03 \pm 2.95$  and  $60.54 \pm 2.83$  cm,  $3092.13 \pm 317.27$  and  $6858.85 \pm 936.91$  gr,  $2224.83 \pm 415.68$  and  $5769.23 \pm 681.63$  gr at birth and 4 months of age, respectively. Based on the ratio test, there was no significant statistical difference between job, education, sunlight exposure and history of breastfeeding in the two mothers' groups ( $P > 0.05$ ), and only a significant difference was observed in supplemental use during pregnancy ( $P = 0.023$ ). As demonstrated in **Table.2**, mean comparison with paired t-test showed that there was a significant difference between the mean level of vitamin D during labor and 4 months after that in both groups of mothers and neonates, at both periods ( $P < 0.001$ ).

**Table-1:** Statistical information of term and preterm neonates and their mothers in this study.

I) Gender of neonates			
Gender	Term Neonates Frequency (%)	Preterm Neonates Frequency (%)	P-value
Boy	17 (56.67%)	12 (40%)	0.439
Girl	13 (43.33%)	18 (60%)	
II) Mean comparison of height (cm) and weight (gr) of term and preterm neonates at birth and age of 4 months			
Variables	Term Neonates Mean (SD)	Preterm Neonates Mean (SD)	
Height at birth (cm)	50.55 (2.32)	46.03 (2.95)	
Height at age of 4 months (cm)	63.00 (2.21)	60.54 (2.83)	
Weight at birth (g)	3092.13 (317.27)	2224.83 (415.68)	
Weight at age of 4 months (g)	6858.85 (936.91)	5769.23 (681.63)	
III) Comparison of frequency of occupation, education, sunlight exposure, supplementary use during pregnancy, and previous breast-feeding history in mothers			
Variables	Mothers of term neonates Frequency (%)	Mothers of preterm neonates Frequency (%)	P-value
Occupation	Housewife	26 (86.66%)	0.192
	Employee	2 (6.67%)	
	Self Employed	2 (6.67%)	
Education	Illiterate	1 (3.33%)	0.561
	Elementary	4 (13.33%)	
	Middle School	5 (16.67%)	
	High School	13 (43.33%)	
	Academic	7 (23.34%)	
Daily sunlight exposure	30 (100%)	29 (96.67%)	0.321
Supplemental use during pregnancy	29 (96.67%)	26 (86.67%)	0.023*
Previous breast-feeding history	19 (63.33%)	22 (73.33%)	0.795

\*Significance level of 5%. SD: Standard deviation.

**Table-2:** Mean comparison of vitamin D level (ng/mL) of term and preterm neonates and their mothers.

Participants	Vitamin D level during labor Mean (SD)	Vitamin D level 4 months after labor Mean (SD)	P-value
Mothers of term neonates	17.13 (8.02)	24.86 (9.84)	P<0.001**
Mothers of preterm neonates	16.25 (7.20)	23.83 (9.15)	P<0.001**
All mothers (n=52)	17.47 (7.40)	24.35 (9.42)	P<0.001**
	Vitamin D level at birth	Vitamin D level at age of 4 months	P-value
Term Neonates	24.53 (10.93)	41.30 (12.87)	P<0.001**
Preterm Neonates	19.54 (8.88)	43.78 (15.51)	P<0.001**
All Neonates (n=52)	22.03 (10.18)	42.54 (14.17)	P<0.001**

\*\*Significance level of 1%. SD: Standard deviation.

The relative frequency of vitamin D levels in mothers of term and preterm infants during labor and four months after were 5, 15, 8, 2 and 2, 8, 9, 7 mothers; 7, 15, 7, 1 and 1, 7, 13, 5 mothers less than 10, between 10 and 20, between 20 and 30, and more than 30 ng/ml, respectively. Based on Chi-square test, no significant difference was found between the two periods in mothers in terms of the relative frequency of vitamin D levels (P=0.865, P=0.691) (**Table.3**). There was also no

significant difference in the relative frequency of vitamin D levels in term and preterm neonates at both periods (birth and 4 months of age) (P =0.467, P=0.408). According to the Pearson test, presented in **Table.4**, there was a significant positive correlation between vitamin D levels of mothers of term and preterm neonates during labor and vitamin D level of their neonates at birth (r =0.858, P<0.001; r =0.744, P<0.001, respectively).

**Table-3:** Comparison of frequency of vitamin D level (ng/mL) on term and preterm neonates and their mothers

Serum Vitamin D Levels (ng/mL)	During labor Frequency (%) (n=60)		4 months after labor Frequency (%) (n=52)	
	Mothers of term neonates	Mothers of preterm neonates	Mothers of term neonates	Mothers of preterm neonates
Less than 10	5 (16.67%)	7 (23.33%)	2 (7.68%)	1 (3.85%)
10<D<20	15 (50%)	15 (50%)	8 (30.77%)	7 (26.92%)
20<D<30	8 (26.67%)	7 (23.33%)	9 (34.62%)	13 (50%)
Higher than 30	2 (6.66%)	1 (3.34%)	7 (23.33%)	5 (19.23%)
Chi-Square test	Chi-Square value= 0.733, df=3, P-value= 0.865		Chi-Square Value= 1.461, df=3, P-value= 0.691	
Serum Vitamin D Levels (ng/mL)	Neonates at birth Frequency (%) (n=60)		Neonates at age of 4 months Frequency (%) (n=52)	
	Term	Preterm	Term	Preterm
Less than 10	5 (16.67%)	6 (20%)	0	1 (3.85%)
10<D<20	8 (26.67%)	12 (40%)	2 (7.69%)	2 (7.69%)
20<D<30	10 (33.33%)	9 (30%)	4 (15.38%)	1 (3.85%)
Higher than 30	7 (23.33%)	3 (10%)	20 (76.93%)	22 (84.61%)
Chi-Square test	Chi-Square value= 2.544, df=3, P-value=0.467		Chi-Square value= 2.895, df=3, P-value=0.408	

SD: Standard deviation.

**Table-4:** Correlation between vitamin D levels of term and preterm neonates and their mothers at birth.

Vitamin D levels of mothers of term neonates during labor	Vitamin D levels of term neonates at birth	
	r	P-value
	0.858	P<0.001**
Vitamin D levels of mothers of preterm neonates during labor	Vitamin D levels of preterm neonates at birth	
	r	P-value
	0.744	P<0.001**

\*\*Significance level of 1%. r= Pearson correlation.

#### 4- DISCUSSION

The aim of this study was to evaluate the effect of vitamin D supplementation on term and preterm neonates and mothers for 4 months. Vitamin D plays an important role in the growth and sustainability of maternal and fetal bone health. Maternal vitamin D deficiency is a major and often undetectable health problem (19). A high ratio of mothers of term and preterm newborns in the present study (mean of  $17.13 \pm 8.02$  and  $16.25 \pm 7.20$  ng/ml) had vitamin D deficiency even after 4 months of intervention (mean of  $24.86 \pm 9.84$  and  $23.83 \pm 9.15$  ng/ml); this ratio was slightly lower in mothers of preterm neonates. Therefore, the results of this study confirm that preterm labor is considered as a risk factor for vitamin D deficiency (20).

Many researches, like our study, have recently defined vitamin D deficiency to be at levels below 30 ng/ml (6, 21, and 22). According to this definition and the results of this study, 93.33% and 96.66% of mothers of term and preterm children had a serum level of vitamin D less than 30 ng/ml at the labor time, respectively. After the intervention, the percentage of mothers with vitamin D deficiency (less than 30 ng/ml) was slightly reduced after 4 months which was 73.08% and 80.77% for mothers of term and preterm infants respectively. In a study conducted by Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences Vitamin D deficiency was shown to fall at the range of 40-80% in Iran (19).

Vitamin D deficiency in pregnant women has been previously reported in several studies (8, 10, 19, 21, 23, 24), and most of these studies have determined vitamin D levels based on its serum levels. In 2013, Hatami et al. (21) reported the prevalence of vitamin D deficiency (less than 20 ng/ml) to be 76% among mothers who had their blood sample taken in the delivery room. In three similar studies conducted in Iran, Maghbooli et al. (25), Kazemi et al. (26), and Salek et al. (27), the prevalence of vitamin D deficiency (less than 20 ng/ml) in pregnant women in Tehran, Zanzan and Isfahan in 2007, 2008, and 2009 was 66.8%, 5.7%, and 86%, respectively. Kazemi et al. (26) indicated that the vitamin D deficiency of pregnant women in winter (86%) was higher during summer (46%). Moreover, although in this study the ratio of vitamin D deficiency level is similar to those domestic studies, the mean serum levels of vitamin D in mothers of term and preterm neonates in the labor time ( $17.13 \pm 8.02$  and  $16.25 \pm 7.20$  ng/ml) was higher than the average serum level of vitamin D of pregnant women in the studies of Maghbooli et al. (25) and Hatami et al. (21). In 2017, Niramitmahapanya et al. (28) reported, the serum vitamin D level of Thai pregnant women was  $22.29 \pm 7.15$  nmol /L during delivery. This amount in the study of Czech-Kowalska et al. (29) was 13.7 and 16.1 ng/ml in both the intervention and control group in 2014, respectively. Unlike our study, there was no difference between the two groups of Czech-Kowalska et al.'s

study (29) in terms of the vitamin D level of mothers at the time of delivery. A study in Greece in 2006 by Nicolaidou et al. (30), showed that 19.5% of mothers had a serum vitamin D level less than 10 ng/ml (severe deficiency) at delivery, which was almost similar to the results in our study for mothers of term (16.66%), and preterm (23.23%) infants at labor time. In 2009, another investigation in Turkey showed 27% of mothers had a severe vitamin D deficiency (31). Srinivasan et al. (32) also found that mothers and their preterm newborns had a vitamin D deficiency (less than 20 ng/ml). Studies in the countries of Turkey, Saudi Arabia, India, Spain and Finland showed that pregnant women do not receive enough vitamin D (19).

Specker (33) in a review study, indicated that higher level of vitamin D in pregnant women has direct association with higher vitamin D consumption. In this study, the vitamin D level of maternal cord blood was also correlated with the intake of sufficient amounts of vitamin D supplements. The prevalence of vitamin D deficiency in Iranian pregnant women is relatively high, and with a cut-off point of less than 30 ng/ml, only about 15% of Iranian pregnant women have normal levels of vitamin D (34). Therefore, it is necessary to provide intervention programs to decrease vitamin D deficiency in Iranian pregnant women.

The prevalence of vitamin D deficiency in pregnant women (<30 ng/ml) in terms of geographical areas in Iran showed that the highest prevalence was observed in central (42.9%), and southern regions (90%) of the country (34). However, it should be noted that there are no comprehensive studies regarding vitamin D deficiency in pregnant women in northern and western parts of Iran (34). Thus, further investigation is recommended to estimate the prevalence and implement the national program accordingly. In fact, based on our results and previous studies in Iran (20, 21,

25), the level of vitamin D in women during labor was lower than that reported in other countries (19, 33). Hence, the use of vitamin D supplements during pregnancy seems to be a good solution. In Iran, the use of vitamin D supplements during pregnancy is not included in ordinary daily care and it is individually prescribed (20). In addition, Islamic clothing and inappropriate diet, can lead to a lower vitamin D levels in women. Disturbances in vitamin D deficiency depend on genetic, ethnic, and nutritional factors. As a result, vitamin D supplements are needed more in areas where there is a shortage, especially during the first year of pregnancy and childhood. Lack of sun exposure, systematic use of sunscreens, living in northern latitudes, dark skin, obesity, senescence, poor nutritional status, absorption syndromes and medications have also been reported as risk factors for vitamin D deficiency (35, 36).

In this study, the vitamin D level of term and preterm infants significantly increased over the 4 months of intervention and reached the normal and even higher levels. The mean vitamin D level of term and preterm newborns surged from  $24.53 \pm 10.93$  to  $41.30 \pm 12.87$  and from  $19.45 \pm 8.88$  to  $43.78 \pm 15.51$  ng/ml, respectively, and the differences were significant ( $P < 0.001$ ). The prevalence of vitamin D deficiency (less than 30 ng/ml) in term and preterm infants was 76.77% and 90% at birth, respectively; while these values were significantly lower after 4 months of intervention and reached 23.07% and 15.39% for term and preterm neonates, respectively. Therefore, the effect of vitamin D intake could clearly be seen after 4 months of intervention, and this effect was stronger in preterm infants. Fallahi et al. (20) suggest that preterm neonates may need more vitamin D than term neonates in the first months of life, since preterm infants are at increased risk



of vitamin D deficiency due to the limited transfer of vitamin D through placenta in the third trimester of pregnancy. In 2018, the prevalence of vitamin D deficiency in Hosseinzadeh et al.'s study (10.) was 93.5% and 6.5% for pregnant women, 94.2% and 3.9% for newborns, respectively. Srinivasan et al. (32), also showed that vitamin D levels in preterm infants was lower than in term ones. They found that supplementation of preterm newborns with 800 to 1000 units of vitamin D have been effective and increased the vitamin D levels above 30 ng/ml. Similar to our results, 90% of newborns had a normal level of vitamin D in Shakiba et al.'s study (37) after 4 months of intervention with Vitamin D supplementation. In 2014, Burriss et al. (38) showed that the chances of vitamin D deficiency (less than 20 ng/ml) in preterm infants less than 32 weeks old were 4.2 times higher than infants less than 37 weeks of age. In line with our results, Hollis et al. (13), and Yang et al. (39) also point out the important role of vitamin D supplements in term and preterm infants. Yang et al. (39) have stated that daily supplementation of vitamin D of 800-1000 IU versus 400 IU in preterm neonates will not only positively affect growth, but also the immune function.

In the present study, vitamin D levels of neonate cord blood were higher than their maternal serum levels, which is consistent with other studies (20, 24, 25, 37, 38). There was a direct and significant correlation between maternal and neonatal vitamin D level in both groups at birth time ( $P < 0.001$ ,  $r = 0.858$  &  $P < 0.001$ ,  $r = 0.744$ ). This means that as the level of vitamin D in mothers increases, the level of vitamin D in the neonates increases as well. Therefore, the level of vitamin D in infants entirely and positively depends on the nutritional status of vitamin D in their mother. In line with these results, Ergür et al. (31) and Özdemir et al. (35) suggested

that maternal vitamin D deficiency is the most important factor in vitamin D deficiency in neonates. Andiran et al. (40) have reported that the main risk factor of low serum vitamin D in infants is a vitamin D level below 10 ng/ml in their mothers. The lactating mothers, with proper vitamin D intake, can completely transfer it from their blood to their milk and supply the adequate vitamin D for the neonates, and in this case, infants may not require any additional supplements (13, 41). In addition, Hollis et al. (13) have pointed out that there were no side effects of vitamin D supplements for breast-fed infants.

#### 4-1. Study Limitations

As one of the limitations of this study was the small sample size of the research and a decrease in the number of participants in the study after four months due to the reluctance to continue the research, as well as the lack of consideration of maternal and neonatal exposure hours to sunlight effect. In the future studies, the different dosage of vitamin D supplements and its effect on mothers and their term and preterm newborns can be investigated at different times of pregnancy, as well as after that.

#### 5- CONCLUSION

According to the results of this study, vitamin D intake is associated with maternal nutritional status. Therefore, vitamin D nutritional modification programs should be considered by promoting the use of high calcium and vitamin D sources, as well as the enrichment and complementarity of mothers and infants at risk. In addition, like other studies in different parts of Iran, mothers and infants have low levels of vitamin D in this study as well. However, the results of this study showed that regular consumption of vitamin D supplements has a positive and significant effect on serum levels of vitamin D and

can increase the level of vitamin D to normal levels in mothers and especially newborns. However, it is necessary to carry out more extensive studies with a larger statistical population by examining other effective parameters, as well as conducting training classes for pregnant women.

**6- CONFLICT OF INTEREST:** None.

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