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Investigating the Effect of Vitamin D Supplementation on Maternal Serum 25(OH) D Levels

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ABSTRACT

Background & aim: Although evidence confirms the importance of vitamin D supplementation in pregnancy, there is still a debate over the adequate daily doses of vitamin D intake. This study aimed to investigate the effect of 400 and 1,000 IU vitamin D/day on maternal serum 25 (OH) D levels.

Methods: This quasi-experimental study was carried out on 74 healthy pregnant women between June 12 and September 22, 2019. The intervention group (n=44) received 1,000 IU vitamin D/day from 8 to 10 weeks of pregnancy for 17 weeks, while the control group (n=39) took multivitamin supplements (400 IU vitamin D/day) from 16 weeks of pregnancy for 12 weeks. Maternal serum 25 (OH) D levels were measured at 25-28 weeks of gestation. Data were analyzed in SPSS software (version 21) through the Chi-square, Student's t-test, Mann-Whitney U, and linear regression tests.

Results: There was no significant difference between the two groups at the beginning of the study in terms of 25 (OH) D concentration (P=0.23). The intake of 1,000 IU vitamin D/day had a significant (β=0.28, P<0.001), yet small effect (effect size=0.30), on increasing serum 25 (OH) D levels after controlling the confounding variables. About half of the females who took 1,000 IU vitamin D/day had a serum 25(OH) D level less than 30 ng/dl at 25-28 weeks of pregnancy.

Conclusion: Even after receiving 1,000 IU/day Vitamin D, vitamin D insufficiency was still prevalent during the second trimester of pregnancy. It seems that a higher dosage of vitamin D is required for pregnant women.

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Introduction

Vitamin D plays an important role in bone mineralization (1); moreover, it is critical for immune function (i.e., anti-inflammatory immune response involved in such processes as fertilization, implantation, and maintenance of pregnancy) (2). New studies have shown that vitamin D status during pregnancy is associated with adverse outcomes of pregnancy, such as recurrent pregnancy loss, induced hypertension, preeclampsia, gestational diabetes mellitus, cesarean section, and postpartum depression (3, 4). The results of studies also revealed that a fetus depends fully on the maternal supply of vitamin D in a way that its deficiency may result in adverse neonatal outcomes, such as abortion, premature birth, low birth weight, intrauterine growth retardation, and neonatal hypocalcemia (5, 6).

It has been claimed that serum 25-hydroxyvitamin D (25[OH] D)>20 ng/ml is adequate for pregnant women; however, there is still debate over this matter, and other studies considered serum 25(OH) D>30 ng/ml an optimal level (4, 7-9). The World Health Organization (WHO) noted that vitamin D deficiency is prevalent among pregnant women. (10) The results of studies conducted in the Middle East showed that despite abundant

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sunshine, hypovitaminosis D (i.e., 25[OH]D level less than 20 ng/ml) is prevalent (54%-90%) among pregnant women (11). Moreover, based on the literature, vitamin D deficiency is common among Iranian pregnant women, and about 42.4%, 55.8%, and 80% of pregnant women have serum 25(OH)D concentrations of less than 10, 20, and 30 ng/ml, respectively (12).

Although sunlight is the best source of vitamin D, clothing habits (wearing hijab in Iran) and the use of sunscreen prevent or minimize the amount of sun exposure in women (13). Furthermore, the dietary sources of vitamin D is limited in Iranian foods (14), and there is still controversy over vitamin D supplementation during pregnancy. The WHO has not recommended the intake of vitamin D supplements as a prenatal routine care; however, it has suggested a daily intake of 200 IU vitamin D only in case of documented vitamin D deficiency (15).

In addition, according to some studies, pregnant women may benefit from a daily intake of 1500-4000 IU vitamin D (4, 16, 17). Based on the Iranian Integrated Maternal Health Care Guideline, daily multivitamin/mineral (MVM) capsules (containing 400 IU vitamin D) are prescribed for pregnant women free of charge from 16 weeks of pregnancy up to the third month following the childbirth (18). Moreover, according to a recent recipe from the Ministry of Health and Medical Education in Iran, pregnant women are advised to consume 1,000 IU vitamin D per day from the beginning of the pregnancy (19).

The WHO has noted that any program has to be monitored and evaluated to make sure about its efficiency and impact (if any). The result help to provide feedback on whether the program is appropriate for the target population or that it should be modified if necessary (20). Review of the related literature revealed that no study has yet addressed and compared the effect of these recommendations on pregnant women's serum 25(OH) D in disadvantaged area, such as Sistan and Baluchestan province, Iran. Therefore, the present study aimed to examine the effect of daily intake of 400 IU and 1,000 IU vitamin D on pregnant women's serum 25(OH) D at 25-28 weeks of pregnancy.

Materials and Methods

This quasi-experimental study was conducted (from June 12 to September 22, 2019) on 44 and 39 healthy pregnant women who were under prenatal care and assigned to intervention and control groups, respectively, in Zahedan, Iran.

Zahedan is the capital of Sistan and Baluchestan, a disadvantaged province in Iran (21), which is located at 29° North latitude. According to a previous study conducted on the general population in Zahedan, 89.0%, 5.7%, and 5.2% of women have serum 25(OH)D concentrations of <20, within 20-30 ng/ml, and 30-150 ng/ml, respectively (22).

The sample size in each group was calculated according to the following formula:

$$\frac{(z1-\frac{\alpha}{2}+z1-\beta)^2*(s1^2+s2^2)}{(\mu 1-\mu 0)^2}$$

According to a study conducted by Asemi, the sample size was estimated at 35 women per group (α=0.05, 1-β=0.90, $z_{1-\alpha/2}$=1.96, $z_{1-\beta}$=1.28, and μ_1=22.04, μ_0=14.21 ng/dl) (23). It is worth mentioning that the standard deviation scores of the vitamin D were estimated at 9.26 and 6.21 ng/dl in the intervention and control groups, respectively. Considering the possible non-response or loss to follow-up (X=33%), the required sample size was multiplied by 100/(100-X)(24); therefore, a total of 104 pregnant women (52 cases per group) were included in the study.

Furthermore, healthy pregnant women (irrespective of their gravida) who were referred to the laboratory for prenatal tests were entered the present study. The inclusion criteria were: 1) singleton pregnancy, 2) no history of mental illness, 3) no addiction to drugs, 4) no history of medical diseases (e.g., diabetes, celiac disease, cardiovascular disease, and infection), smoking, and drug interference (e.g., phenytoin) with vitamin D, and 5) vitamin D intake during the last three months. On the other hand, the women with a baseline level of serum 25(OH) D≤10 or ≥50, diagnosis of medical diseases (e.g., hypertension) during pregnancy, and those who were unwilling to participate and uses other supplements in combination with vitamin D and calcium were excluded from the study.

Initially, due to the lack of simultaneous daily access to all health centers, the sampling

procedure was conducted in Hedayat Central Laboratory in Zahedan, Iran. This governmental laboratory center is open 7 days a week from 7:30 a.m. to 2 p.m. According to the Iranian Integrated Maternal Health Care, laboratory tests (e.g., blood and urine tests) are suggested to all pregnant women at 6-10 weeks and 24-30 weeks of pregnancy (18). Pregnant women from all health centers in Zahedan, Iran, are referred to Hedayat Central Laboratory for prenatal tests.

The study population included all women who were referred to this center from all around the city. In addition, the blood samples taken for the prenatal tests were also used for the measurement of serum 25(OH) D. Therefore, only one blood sampling was performed to avoid imposing extra fees on the participants for transportation.

Based on the Iranian Integrated Maternal Health Care Guideline, pregnant women have been prescribed daily MVM capsules (containing 400 IU vitamin D) from 16 weeks of pregnancy. Therefore, pregnant women in the control group had a gestational age of 16 weeks. Moreover, according to a recent recipe from the Ministry of Health and Medical Education in Iran, pregnant women are advised to consume 1,000 IU vitamin D per day from the beginning of their pregnancy. Therefore, pregnant women with a gestational age 8 to 10 weeks were conveniently recruited in the intervention group. Gestational age was determined based on the last menstrual period or by ultrasound. The aim of the study and follow-up procedure were explained to all eligible participants, and written informed consent was obtained from those who were willing to participate in the study.

The names of the health centers to which the women referred were mentioned in the study. Furthermore, MVM or vitamin D supplements were given to women, and information form (including demographic characteristics, health information, pregnancy information, and body mass index) were completed for them. The administration of vitamin D or MVM supplement and subsequent follow-ups were conducted in coordination with the staff of health centers. The participants with serum levels of 10<25(OH) D <50 were contacted and asked to attend the health centers.

In the intervention group, each women received 1,000 IU vitamin D3/day (Zahravi Pharmaceutical Company, Tabriz, Iran) for 17 weeks (119 days) other than folic acid (400 mcg) taken up to the 16th week of pregnancy. In order to count the pills taken by participants, they were asked to bring unused and empty blister packs from the previous visit. In addition, they were informed about the symptoms of hypervitaminosis, such as fatigue, loss of appetite, excessive thirst, weight loss, dehydration, constipation, irritability, and nervousness. In addition, all participants were given the researcher's telephone number to ask their questions. Moreover, they were asked not to take supplements containing vitamin D other than those prescribed by the health centers (e.g., iron tablets). If the physician prescribed a supplement for the participants, its pictures should have been sent to the researcher to ensure that they did not receive extra vitamin D; however, the participants were free to take multivitamins that were free of vitamin D. Women would have been excluded from the study if they were willing to take additional supplements containing extra vitamin D.

Based on the Iranian Integrated Maternal Health Care, all pregnant women are recommended to receive multivitamin supplements (simple or MVM, containing 400 IU of vitamin D) once a day from 16 weeks of pregnancy (19). At the time of the study, MVM capsules were either not available in the health centers or limited in number due to financial constraints. To address this limitation, MVM capsules (Iran Daru Co., Tehran, Iran) were given to the participants (same capsules to all women) for 12 weeks (90 days). Each MVM capsule contains 5000 IU vitamin A, 400 IU vitamin D3, 60 mg vitamin C, 1.5 mg vitamin B_1, 1.7 mg vitamin B_2, 2 mg vitamin B_6, 6 mcg vitamin B_{12}, 30 IU vitamin E, 20 mg nicotinamide, 0.4 mg folic acid, 18 mg ferrous fumarate, 125 mg calcium carbonate, 0.15 mg potassium iodide, and 100 mg magnesium oxide. All participants were then asked to send a picture of any other multivitamin supplements that were prescribed for them by the physician so that the researcher was ensured that the participants did not receive extra doses of

vitamin D more than what was contained in the prescribed multivitamins.

Hedayat Central Laboratory in Zahedan, Iran, was provided with a list of all women who participated in the study. Afterward, the serum 25(OH) D levels of women who were referred to the laboratory for the second round of prenatal tests were also measured. Following that, fasting blood samples were taken between 7:30 and 9 a.m., and after centrifuging, the serums were stored at -20°C. Eventually, the serum concentration of 25(OH) D was measured (before and at the end of the intervention) using an ELSSA Kit (Pishtaz Teb Zaman Diagnostics, Tehran, Iran).

Data were analyzed in SPSS software (version 21) through the Chi-square, exact Chi-square, and Fisher's exact tests. The normal distribution of the continuous variables, such as serum 25(OH) D level was investigated using the Kolmogorov-Smirnov test. Moreover, a student's t-test (if the data in both groups show a normal distribution) or Mann-Whitney U test (if the data were not normally distributed) was employed to compare the means in the two groups.

Furthermore, multiple linear regression was utilized to estimate the potential determinant of vitamin D levels, including serum 25(OH) D level as a dependent variable, as well as vitamin D supplementation, gravida, and dairy intake per day as independent variables. Dichotomous variables included the use of vitamin D supplements and dairy intake per day (no adequate intake=0 and adequate intake). Moreover, gravida was considered a continuous variable. The ENTER method was used in multiple linear regression to study the role of each independent variable in the 25(OH) D levels. Ln transformation was carried out in this study since the levels of serum 25 (OH) D at 28 weeks of pregnancy did not fit in a normal distribution. For all the analyses, two-tailed tests were utilized to compare the variables in two groups. A p-value less than 0.05 was considered statistically significant.

The magnitudes of differences between the results of the two groups were expressed as a standardized effect size. To calculate the effect size, PASS 15 and G* Power 3.1 software were used in this study. The G* Power covers statistical powers analysis for many different statistics (e.g., t-test, X^2 test, and exact tests). It also can be used to calculate the effect size for parametric and nonparametric tests (25). The effect size was interpreted as trivial (<0.20), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.), and very large (>2.0) (26).

Results

Figure 1 demonstrates the flow diagram of participants. Out of 104 healthy pregnant participants (52 cases per group), seven women in the intervention (n=3) and control groups (n=4) were excluded from the study due to Vitd<10 or Vitd≥50 ng/dl. Based on the obtained data, three women had a serum 25(OH) D level less than 10 (about 9.8 ng/ml).

Accordingly, they were excluded from the study and referred to a gynecologist. Subsequent follow-ups showed that they had not followed the recommendations. The participants were then given 1,000 IU oral vitamin D3 per day for about 17 weeks upon request and due to ethical issues. It should be noted that they were informed about other sources of vitamin D (i.e., sunlight and food). At 28 weeks of pregnancy, their serum 25(OH) D levels were about 29 ng/ml. In addition, four women, who had a serum 25(OH) D level higher than 50 ng/ml were excluded from the study.

Furthermore, 14 women were excluded from the study due to the occurrence of abortion (n=2), reluctance to cooperate in the study (n=4), and inaccessibility (n=8). Eventually, the intervention and control groups included 44 and 39 healthy pregnant women, respectively. The primary analysis of the serum 25(OH)D levels <12, 12≤20, 20≤30, 30≤32, and 32-48 ng/ml (27) in all women (n=74) were 9.5%, 29.7%, 54%, 4.1%, and 2.7%, respectively.

Table 1 tabulates the demographic characteristics of the participants in the two groups and vitamin D status before the intervention. The mean values of the gestational age were obtained at 8.85±1.36 and 13.69±1.30 weeks at the baseline for the intervention and control groups, respectively (P<0.001).

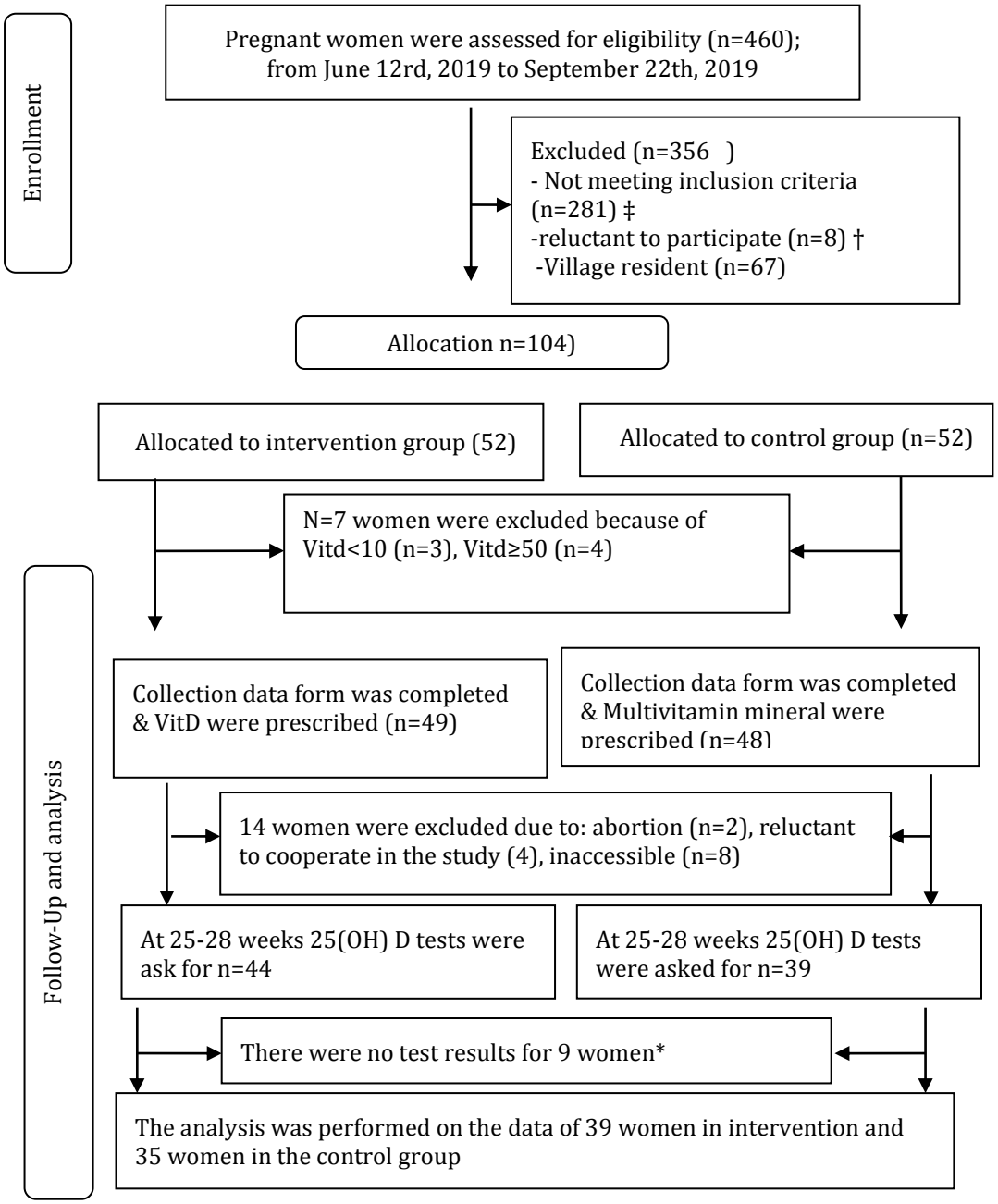

Figure 1. Flow diagram of study participant

Table 1. Comparison of women's characteristics between groups

	Groups		P- Value†
	Intervention (n=39)	Control (n=35)	
women's characteristics	**N (%)**	**N (%)**	
Ethnicity			
Baluch	31(79.5)	29(82.9)	
Fars	8(20.5)	6(17.1)	0.71
Education level			
Illiterate	3(7.7)	7(20)	
Primary school	13(33.3)	16(45.7)	
Secondary school	9(23.1)	2(5.7)	
High school and higher	14(35.9)	10(28.6)	0.07
Exposure to sunscreen			
≤15min	32(82.1)	25(71.4)	
>15min	7(17.9)	10(28.6)	0.27
Use of sunscreen cream			
Yeas	20(51.3)	16(45.7)	
no	19(48.7)	19(54.3)	0.63
Baseline BMI			
<30	30(76.9)	26(74.3)	
≥30	9(23.1)	9(25.7)	0.79
Adequate dairy intake per day‡			
Yes	10(25.6)	18(51.4)	
no	29 (74.4)	17(48.6)	0.02
	Mean(SD)	**Mean(SD)**	
Age (year)	24.23(5.71)	26.11(6.8)	0.2‡
Gravida	2(1.5)	3.08(1.72)	0.002**

‡ Adequate is equal to four servings of dairy products (low-fat milk, low fat yogurt, low fat chees, ice-cream, curd)
† Chi-square ‡t-test ** Mann-Whitney U test

At the baseline, the mean±SD and median (min-max) values of serum 25(OH) D levels were 20.30±5.84 and 21.10 (10.80-31.60) ng/ml, as well as 22.13±7.31 and 22.2 (10.60-48) ng/ml for the intervention and control groups, respectively (P=0.23). The intervention and control groups received 1,000 IU oral vitamin D per day for 17 weeks (since the first trimester) and 400 IU multivitamin mineral capsules (since 1ᵒ weeks of pregnancy) per day for 12 weeks. Furthermore, the mean numbers of taken vitamin D supplements for the intervention and control groups were 111.12±10.44, and 84±7.74, respectively. Afterward, serum 25(OH) D level was measured again at about 25-28 weeks of pregnancy.

Table 2. Comparison of Serum 25(OH) D at baseline and after supplementation between groups

	Groups		P- Value	95% CI		Effect size*
	Intervention (n=39)	Control (n=35)		Lower	Upper	
	Mean(SD)	Mean(SD)				
Baseline serum 25(OH)D ng/ml	20.30(5.84)	22.13(7.31)	0.23†	-1.22	4.88	0.27
Serum 25(OH)D level (ng/ml) at 25-28 weeks of pregnancy	32.54(10.45)	25.61(7.23)	0.002‡	0.001	0.002	0.77
Serum 25(OH)D ng/ml after supplementation minus baseline 25(OH)D level	12.24(10.57)	3.48(10.23)	0.001†	-13.59	-3.92	0.84

†t-test ‡ Mann-Whitney U test * Gpower software was used to calculate effect size

At 25-28 weeks of pregnancy, the mean±SD and median (min-max) values of serum 25(OH)D level were estimated at 32.54±10.45 and 29.20 (16.30-54.10) ng/ml, as well as 25.61±7.23 and 25 (12.30-48.10) ng/ml for the intervention and control groups, respectively (P=0.002; Table 2).

The obtained data showed an increase in the mean serum 25(OH) D level in both groups, compared to that reported at baseline. However, serum 25(OH) D levels in the intervention group (12.24±10.57) were significantly (P=0.001) higher than those measured in the control group (3.48±10.23) after 17 weeks of supplementation (Table 2).

The maximum levels of serum 25(OH) D concentrations were 54.10 and 48.10 ng/ml in the intervention and control groups, respectively.

Table 3. Determinant of serum 25(OH) D level (ng/ml) after supplementation from a multiple linear regression

	Unstandardized coefficient		P- Value	95% CI	
	β	SE		Lower	upper
Constant	3.70	0.18	<0.001	3.33	4.07
Group					
Vitamin D	-0.28	0.07	<0.001	-0.43	-0.13
Multivitamin	0.04	0.02	0.05	-0.001	0.08
Adequate dairy intake per day					
yes					
No	-0.03	0.07	0.60	-0.18	0.10

The results of multiple linear regression indicated that the regression equation fits the data [F=5.02, P=0.003] and that adequate dairy intake per day and gravida were not significant predictors of the serum 25 (OH) D levels at 28 weeks of pregnancy. Furthermore, the R^2 value (R^2=0.177) in the model summary indicated that the model explains only 17% of the variance of the dependent variable (serum 25 [OH] D) at 25-28 weeks by independent variables included in the linear model (Table3).

Table 4. Maternal vitamin D status before and after the intervention between groups

serum 25(OH)D level	Groups		P-Value	Effect size*
	Intervention (n=39)	Control (n=35)		
	N (%)	N (%)		
Baseline:				
<20 (deficiency)	16(41.0)	13(37.2)	0.70‡	0.11
20-29 (insufficiency)	21(53.9)	18(51.4)		
≥30 (Sufficient)	2(5.1)	4(11.4)		
After intervention:				
<20 (deficiency)	4(10.2)	7(20.0)		
20-29 (insufficiency)	12(30.8)	18(51.4)	0.03†	
≥30 (Sufficient)	23(59.0)	10(28.6)		0.30

† Chi-square ‡Fisher Exact test * PASS 15

Table 4 shows that after the intervention, 59% and 28.6% of women in the intervention and control groups had a sufficient level of vitamin D (serum 25 [OH]D ≥30 ng/ml) at 25-28 weeks of gestation, respectively (P=0.03). Meanwhile, 50% and 71.4% of women in the intervention and control groups had a deficient or insufficient level of vitamin D (serum 25 [OH] D less than 30 ng/ml) at 25-28 weeks of gestation, respectively. No adverse event associated with vitamin D occurred among participants.

Discussion

This study aimed to examine the effect of 1000 IU vitamin D/day on maternal serum 25(OH) D levels, compared to 400 IU vitamin D/day. The results revealed that the intake of 1,000 IU vitamin D/day had an insignificant effect on the improvement of maternal serum 25(OH) D level by 25-28 weeks of pregnancy. Meanwhile, about half of the women who took 1,000 IU vitamin D/day did not achieve serum 25(OH) D levels of 30 ng/mL.

Based on the results, vitamin D deficiency (serum 25[OH] D<20 ng/ml) and insufficiency (serum 25[OH] D<30 ng/ml) rates were obtained at 36.2% and 54%, respectively, among pregnant women in this deprived area during summer. The results of the current study are consistent with the findings of prior reports from Iran (12), the Middle East, and North Africa (11). This demonstrated that vitamin D deficiency and insufficiency were reported in 55.84% and 80.82% of healthy pregnant women, respectively. According to the results of another study performed in Iran, vitamin D deficiency increases during the winter and exists in 86% of Iranian pregnant women (28). This can explain the lower vitamin D deficiency in this study which was conducted during the summer.

It is worth mentioning that skin pigmentation, Hijab wearing to cover the whole body according to the religious and cultural rules, low socioeconomic status, low vitamin D dietary intake, and low physical activity (1, 11, 29) lower the chance of receiving vitamin D through natural ways. All of the aforementioned data support the fact that Iranian pregnant women require to take vitamin D supplements during pregnancy (29, 30).

The obtained results indicated that although the prenatal use of multivitamin (400 IU vitamin D/day) helped to increase serum 25(OH)D levels, compared to that reported at the baseline, almost a quarter of women had a serum 25(OH)D level of less than 20 ng/ml after about three months of supplementation. In the same vein, half of the women had a serum 25(OH)D level of less than 30 ng/ml (vitamin D insufficiency) after about three months of supplementation. Therefore, in line with the findings of the previous studies, the present study revealed that an intake of 400 IU vitamin D/day in pregnant women is inadequate due to its slight effect on maternal serum 25(OH)D level (30, 31).

Furthermore, the intake of 1,000 IU vitamin D/day for 17weeks improved serum 25(OH) D more effectively, compared to 400 IU vitamin D/day. However, the effect size revealed a moderated magnitude of difference between the results obtained from both groups. Inconsistent with previous studies (32), the present study showed that about half of the women who received 1,000 IU vitamin D/day had a serum 25(OH)D level less than 30 ng/dl. There are controversies regarding the adequate or optimal serum level of 25(OH) D during pregnancy. It should be noted that based on the results obtained from the present study, 25(OH) D>20 ng/ml is an adequate dose of vitamin D; however, recent studies have suggested that the optimal level should be higher than 30 ng/ml (7, 30, 33).

The US Endocrine Society noted that the supplementation of at least 1,500-2,000 IU vitamin D/day might be required to achieve serum 25(OH)D>30 ng/dL during pregnancy and satisfy the increased needs of mother and fetus (5, 34). However, according to the novel evidence, the intake of at least 4000 IU vitamin D/day has been recommended, and it has been explained that this dose lies within the safe intake level. In addition, no adverse effects were found regarding the use of the aforementioned dose (35).

The results of the present study are of significant importance since they provide evidence on the recommendation of 1,000 IU vitamin D/day supplements for pregnant women in a deprived area. Although the findings of the current study provided research-based evidence for policymakers and local managers regarding vitamin D supplementation in pregnant women, there were several limitations associated with this study. Initially, due to time constraints, the researchers were unable to follow pregnant women up to the end of their pregnancy and measure their 25(OH) D serum concentrations during the third trimester of pregnancy. Other restrictions regarding this study were that neonatal serum 25(OH) D level was not measured, and the sample size was rather small due to financial and technical

constraints. Finally, the results cannot be generalized to all other cities and rural areas in Iran.

Conclusion

Based on the results, the high prevalence of vitamin D deficiency and insufficiency among pregnant women revealed that preventive strategies should be adopted in this regard. However, supplementation with 1,000 IU vitamin D/day had a moderate effect on improving maternal serum 25(OH) D levels at the second trimester of pregnancy. Meanwhile, the findings of the present study highlighted that even with the supplement of 1,000 IU vitamin D/day, about two-thirds of women had a serum 25(OH) D level less than 30 ng/dl at 25-28 weeks of pregnancy. Therefore, it is recommended that large-scale studies with a higher dose of vitamin D supplementation be carried out to provide robust evidence for the policymakers regarding the safe and effective dose of vitamin D during pregnancy.

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Conflicts of interest

Authors declared no conflicts of interest.

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