Effectiveness of Prenatal Vitamin D Deficiency Screening and Treatment Program: A Stratified Randomized Field Trial

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Context: Despite evidence on the association between hypovitaminosis D and adverse pregnancy outcomes and the positive impact of vitamin D supplementation, no evidence exists supporting a universal screening program in pregnancy as part of routine prenatal care.

Objective: We sought to determine the effectiveness of a prenatal screening program on optimizing 25-hydroxyvitamin D [25(OH)D] levels and preventing pregnancy complications. Also, to identify a safe regimen, we compared several regimens in a subgroup of vitamin D–deficient pregnant women.

Design: Two cities of Masjed-Soleyman and Shushtar from Khuzestan province, Iran, were selected as the screening and nonscreening arms, respectively. Within the screening arm, a randomized controlled trial was conducted on 800 pregnant women.

Setting: Health centers of Masjed-Soleyman and Shushtar cities.

Patients or Participants: Pregnant women aged 18 to 40 years.

Intervention: Women with moderate [25(OH)D, 10 to 20 ng/mL] and severe [25(OH)D, $<$10 ng/mL] deficiency were randomly divided into four subgroups and received vitamin D_3 (D3) until delivery.

Main Outcome Measure: Maternal concentration of 25(OH)D at delivery and rate of pregnancy complications

Results: After supplementation, only 2% of the women in the nonscreening site met the sufficiency level ($>$20 ng/mL) vs 53% of the women in the screening site. Adverse pregnancy outcomes, including preeclampsia, gestational diabetes mellitus, and preterm delivery, were decreased by 60%, 50%, and 40%, respectively, in the screening site. A D3 injection in addition to monthly 50,000 IU maintenance therapy contributed the most to achievement of sufficient levels at delivery.

Conclusions: A prenatal vitamin D screening and treatment program is an effective approach in detecting deficient women, improving 25(OH)D levels, and decreasing pregnancy adverse outcomes. (***J Clin Endocrinol Metab*** **103: 2936–2948, 2018)**

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AEPN, absolute excess probability of vitamin D normalization; D3, vitamin D_3; GLM, generalized linear model; GDM, gestational diabetes mellitus; NNS, number needed to screen; OR_{CMH}, Cochran–Mantel–Haenszel OR; RCT, randomized controlled trial.

Hypovitaminosis D during pregnancy is a well-documented global health concern even in the low-latitude areas with adequate sunlight and dietary intake. Despite lacking population representative data, a suboptimal vitamin D level is estimated to have the highest prevalence in the Middle East (70% to 90%) (1). Evidence from non-interventional studies mostly indicates an inverse association between vitamin D deficiency and pregnancy complications. A systematic review pooling the results of 31 studies shows that insufficient serum levels of 25-hydroxyvitamin D [25(OH0D] are associated with gestational diabetes mellitus (GDM), preeclampsia, small-for-gestational-age infants, an increased risk of bacterial vaginosis, and low birth weight infants (2). However, we have to keep in mind that the observational nature of data can seriously influence the causality inference in this regard.

There are a limited number of interventional studies that have been conducted assessing the effect of vitamin D supplementation on maternal 25(OH)D concentration or adverse maternal and neonatal outcomes (3, 4); however, as data are heterogeneous in terms of definitions, dosing, and primary endpoints, inconsistent results have been obtained from these trials. The recent Cochrane Review inspected the efficacy and safety of gestational vitamin D supplementation (alone or combined with calcium) on maternal and neonatal outcomes (5); pooling the results of trials with moderate quality of evidence are in favor of beneficial effects of supplementation on reduction of preterm delivery and low birth weight with controversial result in term of GDM. Another recent meta-analysis of vitamin D pregnancy randomized controlled trials (RCTs) found no evidence that vitamin D_3 (D3) supplementation provided any protection from complications of pregnancy (6).

At present, no consensus is available on the target concentration of circulating 25(OH)D as the main marker of vitamin D status during pregnancy (7, 8). Whereas the National Academy of Medicine (formerly called the Institute of Medicine) considers 25(OH)D >20 ng/mL as adequate in pregnancy (7), the US Endocrine Society recommends a higher threshold of 30 ng/mL as an optimal level (9). The same controversy applies to the required supplementation dosing to compensate for the deficiency (7, 9, 10).

However, given the uncertainty surrounding the beneficiary impact of supplementation and the optimal dosing required (5), we cannot currently put forth any recommendation for or against a universal screening program as a part of routine antenatal care. Taken together, it seems that running large well-designed RCTs is imperative to determine whether strategies to optimize maternal 25(OH)D levels are effective in improving maternal and neonatal outcomes. Moreover, in a broader aspect, assessment of screening programs can help policy makers to implement the best strategies for resolving the problem. To the best of our knowledge, there is no interventional study comparing screening vs nonscreening regimens for detection and treatment of vitamin D deficiency.

In the current study, we designed a large field trial composed of screening (n = 900) and nonscreening sites (n = 900) among a healthy cohort of pregnant women to examine whether universal screening can improve the pregnancy outcomes in terms of preeclampsia, GDM, and preterm delivery. To further assess the efficacy of different regimens of D3 replacement therapy on improving 25(OH)D levels, in the screening arm we compared four different D3 protocols in subgroups of women with moderate (n = 400) and severe (n = 400) deficiency, compared with normal controls.

Methods

This study was conducted in two phases (Fig. 1). In the first phase, using stratified multistage cluster sampling with a probability proportional to size method, 1600 and 900 first trimester pregnant women attending health centers of Masjed-Soleyman and Shushtar, Khuzestan province, Iran, were recruited for the purpose of the current study. These women had to meet eligibility criteria, including age range 18 to 40 years, gestational age <14 weeks, singleton pregnancy, not consuming multivitamins containing >400 IU/d of D3, and no previous history of chronic diseases.

In the second phase of this study, Masjed-Soleyman participants were assigned to a screening program, and participants of Shushtar were followed as the nonscreening arm. The details of the study procedure have been reported before (11). In brief, in the first phase following enrollment into the study, a fasting blood sample was collected from all participants and transferred to the central laboratory of Masjed-Soleyman; serum samples of participants in Shushtar were stored and kept frozen at −80°C until assayed at the end of the study, whereas vitamin D status of Masjed-Soleyman participants was immediately determined.

Owing to the cost and complexity of the process, 800 pregnant women with vitamin D deficiency from Masjed-Soleyman were randomly allocated to one of the designed intervention programs. The remaining women deficient in vitamin D were referred to specialists for further treatments. Comparison of the basic confounders between the initial recruited sample in Masjed-Solayman and the women who were allocated to intervention indicated no statistically significant difference and hence no selection bias occurred during the allocation of treatment.

Vitamin D administration was initiated 4 to 8 days after the first prenatal visit. Participants from Shushtar and those with serum vitamin D >20 ng/mL from Masjed-Soleyman served as controls. All participants received standard prenatal care, and both maternal and neonatal outcomes were recorded. Gestational age was calculated according to the first day of their last menstrual cycle for women with regular cycles and/or ultrasonography for those with irregular cycles or those who could not precisely recall their last menstrual cycle (n = 178).

In this study the primary outcome for assessment of the screening vs nonscreening arms were preterm delivery (birth at <37 weeks),

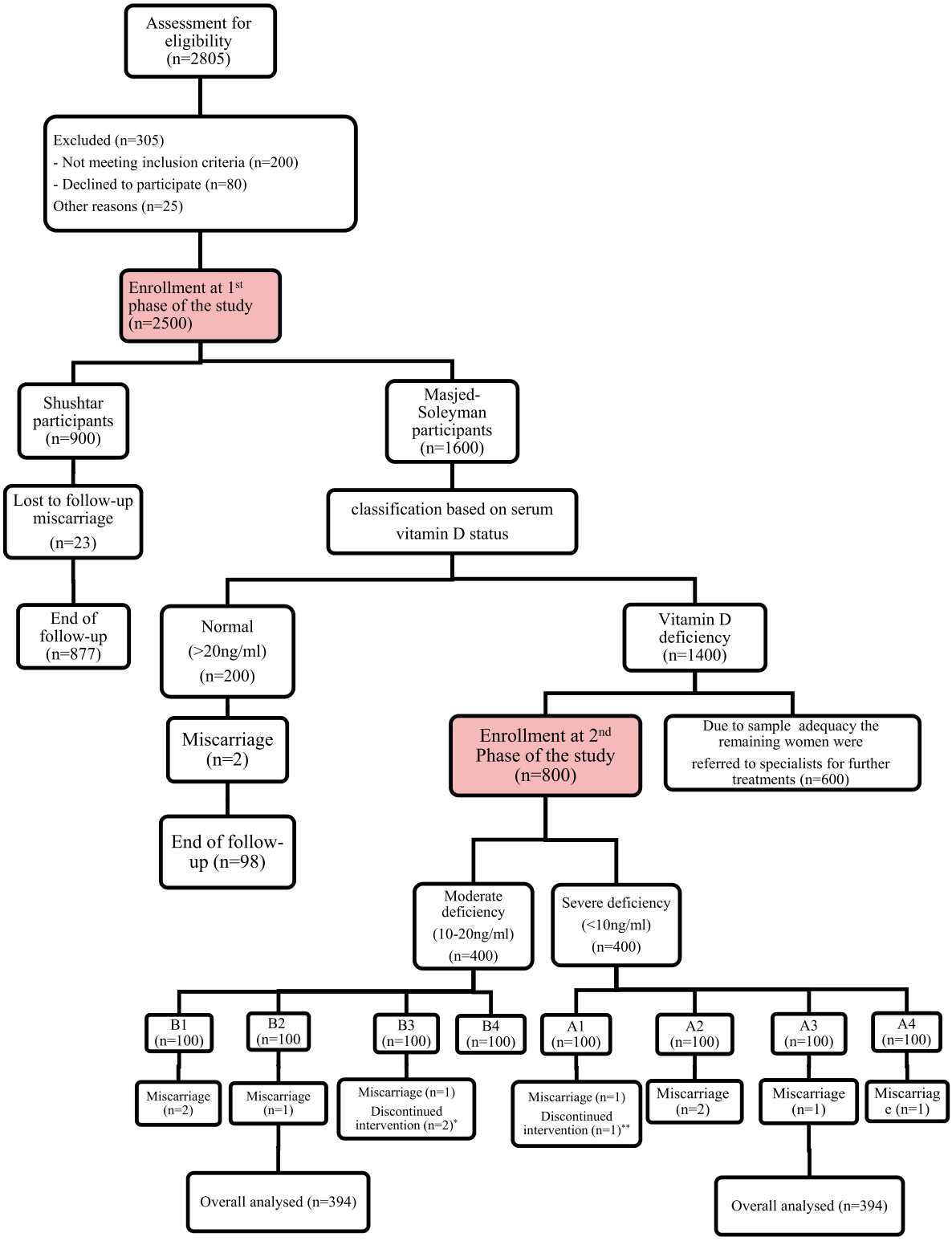


Figure 1. Study flow diagram. Group A1/B1: treated with 50,000 IU of oral D3 weekly for a total duration of 12 wk. Group A2/B2: treated with 50,000 IU of oral D3 weekly for a total duration of 12 wk plus a monthly maintenance dose of 50,000 IU of D3 until delivery. Group A3/B3: treated with intramuscular administration of 300,000 IU of D3 each 6 wk for two doses. Group A4/B4: treated with intramuscular administration of 300,000 IU of D3 each 6 wk for 2 doses plus monthly maintenance dose of 50,000 IU of D3 until delivery. *Discontinued intervention due to car accident and humerus injury (n = 1) and dislike to continue D3 supplementation weeks after consumption (n = 1). **Discontinued intervention due to husband death and subsequent mental problems.

preeclampsia (systolic blood pressure >140 mm Hg or diastolic blood pressure ≥90 mm Hg and 24-hour proteinuria ≥0.3 g, started at >20 weeks), and GDM (glucose intolerance first detected during pregnancy using criteria of the International Association of the Diabetes and Pregnancy Study Groups). Additionally, variables of composite adverse pregnancy outcomes were defined as preeclampsia and/or GDM and/or preterm delivery, and the primary outcome for assessment of the effectiveness of different regimens of D3 replacement therapy was serum concentration of 25(OH)D at delivery.

Randomization and masking

Randomization was performed in blocks of four using a computer-generated list. Physicians, who participated in various phases of the study, were blinded to grouping of women; only the midwife, who did not participate in any phase of the study, was aware of the group that each patient was in. Masking to treatment allocation was not possible, and only those health care workers who determined pregnancy outcomes were blinded to treatment allocation.

Interventions

These women were classified into three groups according to their serum concentration of 25(OH)D as severely deficient (<10 ng/mL), moderately deficient (10 to 20 ng/mL), and >20 ng/mL (6). Those women with severe and moderate vitamin D deficiency were randomly allocated to one of eight interventions (I) and treated as follows:

Subjects with moderate deficiency

I1: 50,000 IU of oral D3 weekly for a total duration of 6 weeks
I2: 50,000 IU of oral D3 weekly for a total duration of 6 weeks and then a monthly maintenance dose of 50,000 IU of D3 until delivery
I3: A single dose of intramuscular administration of 300,000 IU of D3
I4: A single dose of intramuscular administration of 300,000 IU of D3 and then a monthly maintenance dose of 50,000 IU of D3 until delivery

Subjects with severe deficiency

I5: 50,000 IU of oral D3 weekly for a total duration of 12 weeks
I6: 50,000 IU of oral D3 weekly for a total duration of 12 weeks and then a monthly maintenance dose of 50,000 IU of D3 until delivery
I7: Intramuscular administration of 300,000 IU of D3; two doses for 6 weeks
I8: Intramuscular administration of 300,000 IU of D3; two doses for 6 weeks, followed by a monthly maintenance dose of 50,000 IU of D3 until delivery

Oral D3 50,000 IU or cholecalciferol tablets were manufactured by Roche Pharmaceutical (Tehran, Iran) and dispersed in Iran by Zahravi (Tehran, Iran). Intramuscular D3 injection of a 1-mL ampule of 300,000 IU/mL of D3 in sesame oil was manufactured by Caspian Pharmaceutical (Gilan, Iran). Adherence to the supplementation regimen was measured by maternal self-report and pill counts at each prenatal visit. The number of pills returned was divided by the expected number of pills that would have been taken to create a percentage that indicates the adherence of medication regimen.

Circulating 25(OH) levels were measured using the ELISA method and a kit of Immunodiagnostics Systems by AutoAnalyzer (Human Corporation, Germany). This 25(OH)D assay is Food and Drug Administration cleared for clinical use in the United States. The interassay and intra-assay coefficients of variation were 3.891% and 3.37%, respectively (sensitivity of 5 nmol/L). Calibration of the instruments was done as per the manufacturer's instructions, and validation studies were done prior to the test. Samples were analyzed by a single technician using the same equipment throughout the study in a reference laboratory and were measured according to standard operating procedures.

Sample size calculation

Sample size of this study was estimated for two phases accordingly:

1. Screening phase in two sites of Masjed-Soleyman and Shushtar

A cluster sampling method, with a probability proportional to size procedure was assigned. The sample size in Masjed-Soleyman was calculated using the following formula and assumption, resulting in 1537 subjects:

$$n \geq \frac{z^2_{1-\alpha/2}(1-P)}{\varepsilon^2 P} \begin{array}{l} \alpha = 0.05 \Rightarrow z_{1-\alpha/2} = 1.96 \\ P = 0.10 \\ \varepsilon = 0.15 \end{array}$$

The same steps (except for $\varepsilon = 0.2$) were used for calculation of sample size in Shushtar, resulting in 900 subjects. Using the cluster sampling method, 1600 and 900 first trimester mothers were selected from among those receiving prenatal care in health centers in urban regions of Masjed-Soleyman and Shushtar, respectively. Because the prevalence of the specified event was untreated or unrecognized and the number of people who had the risk factor (P) was high in the population, the sample size needed for screening was considered sufficient (α = type one error considered as 0.05; β = type two error ($1 - \beta$ = power) considered as 0.2; P = minimum prevalence of the studied maternal/neonatal events in the population; and ε = error).

2. To compare different regimens in the screening group, the sample size in each group was calculated according to the following formula:

$$n \geq \frac{(z_{\alpha/2} + z_\beta)^2 \sigma^2 (1 + 1/k)}{\varepsilon^2}$$
$$\alpha = 0.05 \Rightarrow z_\alpha = 1.96$$
$$1 - \beta = 0.90 \Rightarrow z_\beta = 1.28$$
$$\varepsilon = \mu_1 - \mu_2$$
$$k = 1$$
$$\theta = \textit{effect size} = |\varepsilon|/\sigma = 0.50$$
$$n = 2(1.96 + 1.28)^2 \left(\frac{1}{0.50}\right)^2 = 84$$

Considering a loss to follow-up of 10%, a total of 100 women in each study group was adequate [α = type one error considered as 0.05; β = type two error ($1 - \beta$ = power) considered as 0.2; ε = the difference between means in groups one

and two; σ^2 = Variane; k = the proportion of two groups' sample size (here k is equal to 1, meaning that the two groups have equal sample size); θ = effect size, which is equal to ε/σ].

Ethical considerations

Written informed consent was obtained from all participants, and the study was approved by the Ethics Committee of the Research Institute of Endocrine Sciences (approval no. 10ECRIES25/10/92). This study is registered in the Iranian Registry of Clinical Trials (code no. IRCT2014102519660N1).

Statistical analysis

Continuous variables with nonnormal probability distribution are presented as median and interquartile range and have been compared by a Mann-Whitney test. Categorical variables are reported by number of positive events (%), separated based on extent of 25(OH)D deficiency. A χ^2 test [for each level of 25(OH)D deficiency] and adjusted Cochran–Mantel–Haenszel test (which control for vitamin D levels) have been applied to determine significant differences. To estimate how the screening regimen influenced all maternal outcomes, compared with nonscreening, common Cochran–Mantel–Haenszel OR (OR_{CMH}) was calculated by the Cochran–Mantel–Haenszel method.

Primary analysis was based on the intention-to-treat principle comparing the odds of maternal pregnancy outcomes in screening vs nonscreening study sites. A generalized linear model (GLM) was applied to estimate the effect of intervention on maternal pregnancy outcomes such as preeclampsia, GDM, and preterm labor. ORs with 95% CIs were calculated by logistic regression (GLM with logit link function). The linear probability model (GLM with identity link function) was used to further estimate number needed to screen (NNS) (as the reciprocal of the absolute risk reduction).

Within the screening arm, the impact of different supplemental regimens on 25(OH)D values were explored. To assess the efficacy of treatment on the risk factor (namely vitamin D deficiency), 25(OH)D levels of >20 ng/mL at delivery were considered as the outcome. Because there existed sparse data (0 cases in Table 4), we estimated the conditional probability of having 25(OH)D level of >20 ng/mL at delivery instead of OR and calculated absolute excess probability of vitamin D normalization (AEPN) with 95% CI.

Results

Descriptive statistics, including characteristics at baseline and delivery according to the screening program, are shown in Table 1. There was no significant difference in baseline characteristics of the participants of the study sites (screening vs nonscreening). Although median baseline maternal 25(OH)D concentration was similar in the screening and nonscreening groups [11 (7 to 16) ng/mL vs 11 (7 to 16) ng/mL; $P = 0.95$], it was significantly higher in the screening vs nonscreening group at delivery [21 (18 to 25) ng/mL vs 11 (7 to 18) ng/mL; $P < 0.001$]. Following supplementation, 2.7% of women in the screening site achieved 25(OH)D levels >30 ng/mL, whereas no increment >30 ng/mL was observed in our nonscreening site.

In the screening site, preeclampsia occurred in 7% and 8% of women with baseline moderate and severe deficiency, respectively, who received one of the different scheduled regimens of D3 replacement therapy; in the nonscreening site, it was observed in 13% and 23% in women with moderate and severe vitamin D deficiency, respectively ($P < 0.001$). After supplementation, prevalence of preeclampsia was estimated as 1.3%, 16.0%, and 38.5% in the normal, moderate deficiency, and severe deficiency groups at delivery, respectively. There was no significant difference in outcomes of preeclampsia between participants with 25(OH)D levels of >20 ng/mL of screening and nonscreening sites (12% vs 6%; $P = 0.16$). No cases of preeclampsia or GDM were detected in women who achieved 25(OH)D levels >30 ng/mL. Comparisons of other pregnancy outcomes are shown in Table 1.

Effects of the screening vs nonscreening program on maternal outcomes in women with moderate and severe deficiency are illustrated in Table 2; screening reduced the risk of preeclampsia by 60% (OR, 0.40; 95% CI, 0.30 to 0.60), and NNS was estimated at 11 (95% CI, 8 to 17), indicating that by screening 11 cases, 1 case of preeclampsia would be prevented. The screening program was associated with a 50% reduced risk [OR, 0.50 (95% CI, 0.34 to 0.88) for GDM; NNS, 50 (95% CI, 2 to 167)], a finding demonstrating that to prevent 1 case of GDM, a minimum of 50 subjects need to be screened. Following implementation of the screening program, the risk of preterm delivery decreased by 40% (95% CI, 0.40 to 0.80), and NNS was estimated at 20 (95% CI, 13 to 50). Subgroup analysis (multiplicative interaction effect) revealed that supplementation in severely vs moderately deficient groups for preeclampsia and GDM did not differ significantly. Subgroup analysis demonstrated a significant difference between moderate and severe groups in terms of supplementation effect for preterm delivery, showing that supplementation in the severely vs moderately deficient groups ($OR_{severe}/OR_{moderate}$) caused a 0-fold to 5-fold statistically significant decrease in the risk of preterm delivery ($P = 0.02$); following supplementation, risk of preterm delivery decreased by 30% (NNS of 50) in moderately deficient groups and 67% (NNS of 8) in severely deficient groups.

Common OR_{CMH} showed that the odds of composite adverse pregnancy outcomes (preeclampsia and/or GDM and/or preterm delivery) in moderately and severely deficient groups of the screening site was 0.45 (95% CI, 0.36 to 0.55) compared with the nonscreening site; that is, screening in these women decreased the odds of adverse events by 55% (depicted as composite variable in subgroup analysis and forest plot in Table 2).

Table 1. Baseline and At-Delivery Characteristics of Study Participants Categorized by Study Sites and Maternal Outcomes

Characteristics	Screening Site (Masjed-Soleyman) (n = 900)	Nonscreening Site (Shushtar) (n = 900)	Overall (n = 1800)	*P* Value[a]
Baseline characteristics				
Age, y	29 (25–32)	29 (25–32)	29 (25–32)	0.62
Marriage age, y	20 (18–22)	19 (17–23)	19 (17–22)	0.07
First delivery age, y	20 (18–22)	20 (18–21)	20 (18–22)	0.57
First pregnancy age, y	21 (19–24)	20 (18–24)	21 (19–24)	0.08
Gestational age	10 (9–12)	10 (9–12)	9 (10–12)	0.07
Gravity	2 (1–3)	2 (1–3)	2 (1–3)	0.95
Parity	1 (0–2)	1 (0–2)	1 (0–2)	0.85
Number of abortions	0 (0–0)	0 (0–0)	0 (0–0)	0.95
Number of children	1 (0–2)	1 (0–2)	1 (0–2)	0.78
25(OH)D (ng/mL)	11 (7–16)	11 (7–16)	11 (7–16)	0.95
Normal	23 (21–27)	22 (21–24)	22 (21–25)	0.07
Moderate deficiency	14 (12–17)	13 (11–16)	14 (11–16)	0.25
Severe deficiency	6 (5–8)	7 (5–8)	6 (5–8)	0.84
SBP, mm Hg	115 (110–120)	120 (110–120)	120 (110–120)	0.86
DPB, mm Hg	70 (60–70)	70 (70–70)	70 (60–70)	0.64
Maternal weight, 6–10 wk of gestation	65 (59–70)	64 (59–70)	64 (59–70)	0.98
At-delivery characteristics				
SBP, mm Hg	116 (111–121)	121 (116–126)	119 (114–124)	0.00
DPB, mm Hg	68 (63–73)	73 (68–78)	71 (68.50–73.50)	0.00
Maternal weight, mo 9	76 (70.25–81.75)	74 (68.50–79.50)	75 (69.50–80.50)	0.00
25(OH)D, ng/mL	21 (18–25)	11 (8–17)	17 (11–22)	0.00
Normal, >20 ng/mL	21 (18–24)	22 (20–24)	21 (19–24)	0.00
Moderate deficiency, 10–20 ng/mL	20 (17–25)	13 (10–16)	16 (12–21)	0.00
Severe deficiency (< 10ng/mL)	22 (17–25)	7 (5–9)	14 (7–22)	0.00
Maternal pregnancy outcomes				
Preeclampsia				0.00[b]
Normal	12 (12)[c]	9 (6)[c]	21 (9)[c]	0.16
Moderate deficiency	29 (7)[c]	54 (13)[c]	83 (10)[c]	0.00
Severe deficiency	35 (8)[c]	75 (23)[c]	110 (15)[c]	0.00
Total	76 (9)[c]	138 (16)[c]	214 (12)[c]	0.00
GDM				0.02[b]
Normal	6 (6)[c]	6 (4)[c]	12 (5)[c]	0.37
Moderate deficiency	11 (3)[c]	17 (4)[c]	28 (4)[c]	0·20
Severe deficiency	17 (4)[c]	30 (9)[c]	47 (7)[c]	0.00
Total	12 (5)[c]	28 (4)[c]	87 (5)[c]	0.02
Preterm delivery				0.00[b]
Normal	12 (12)[c]	9 (7)[c]	21 (9)[c]	0.10
Moderate deficiency	29 (7)[c]	40 (10)[c]	69 (9)[c]	0.14
Severe deficiency	33 (8)[c]	71 (22)[c]	104 (14)[c]	0.00
Total	74 (8)[c]	120 (14)[c]	194 (11)[c]	0.00
25(OH)D levels attained				0.00[b]
Normal	53 (54)[c]	94 (67)[c]	147 (63)[c]	0.00
Moderate deficiency	197 (50)[c]	11 (3)[c]	208 (26)[c]	0.00
Severe deficiency	218 (55)[c]	0 (0)[c]	218 (30)[c]	0.00
Total	468 (53)[c]	105 (12)[c]	573 (33)[c]	0.00

Data are presented as median [interquartile range (Q1 to Q3)] unless indicated otherwise.

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.

[a]Obtained from Mann-Whitney test and χ^2 test for categorical outcomes. Significance level was considered <0.05.

[b]Cochran–Mantel–Haenszel test is used to determine the difference between prevalence of outcome in study sites controlling for 25(OH)D levels.

[c]Frequency (%).

Results showed that for a case with moderate vitamin D deficiency at baseline who received treatment, compared with the same case but unsupplemented, the AEPN was 0.47-fold (95% CI, 0.42 to 0.52) and 0.55-fold (95% CI, 0.50 to 0.61) higher for pregnant women with moderate and severe deficiency, respectively (Table 3).

Box plots for serum concentrations of vitamin D levels at delivery per protocols of interventions (for screening site), as well as various groups of vitamin D deficiency in the nonscreening site (normal, moderate, and severe vitamin D deficiency), in addition to the maternal 25(OH)D sufficiency levels in the nonscreening site (Shushtar) at

Table 2. Maternal Outcomes Classified According to Study Sites and Baseline 25(OH)D Levels

Outcomes		N(%) Screening site (Masjed-Soleyman)[a]	N(%) Non-screening site (Shushtar)[aa]	OR (95% CI) (p-value)[b]	OR (95% Cl)	NNS (95% CI)	P-value for interaction effect
	Moderate deficiency	29(7)	54(13)	0·5(0·3-0·8) (0·01)*		17(10-50)	
Pre-eclampsia	**Severe deficiency**	35(8)	75(23)	0·3(0·2-0·5) (<·001)*		7(5-11)	0·15
	Total	64(8)	129(17)	0·4(0·3-0·6) (<0·001)		11(8-17)	
	Moderate deficiency	11(3)	17(4)	0·7(0·3-1·4) (0·30)		100(-25-100)	
GDM	**Severe deficiency**	17(4)	30(8)	0·5(0·3-0·9) (0·02)		20(13-125)	0·42
	Total	28(4)	47(6)	0·5(0·3-0·9) (0·01)*		50(2-167)	
	Moderate deficiency	29(7)	40(9)	0·7(0·5-1·2) (0·24)		50(-50-17)	
Preterm delivery	**Severe deficiency**	33(7)	71(20)	0·3(0·2-0·5) (<·001)*		8(5-13)	0·02*
	Total	62(8)	111(15)	0·6(0·4-0·8) (<·001)*		20(13-50)	
	Moderate deficiency	62(16)	94(23)	0·6(0·4-0·9) (0·01)*		20(13-55)	
Composite adverse pregnancy outcomes**	**Severe deficiency**	71(18)	129(39)	0·3(0·2-0·5) (<·001)*[c]		8(5-13)	<0·001*
	Total	133(17)	223(29)	0·45(0·36-0·55) (<·001)*[c]	0 0·5 1·0 1·5	12(9-18)	

[a]Number of positive events in subgroups of 25(OH)D levels in intervention site
[aa]Number of positive events in subgroups of 25(OH)D level in no intervention site
[b]Odds ratio for pre-eclampsia, GDM and preterm delivery (p-value obtained from logistic regression model)
[c]OR_{CMH}: Cochran Mantel Haenszelcommon Odds Ratio

P-value obtained from subgroup analysis: the effect of intervention on the severe group compared to moderate group
*significance level was considered <0·05
** pre-eclampsia and/or GDM and/or preterm delivery
25(OH)D; 25-hydroxyvitamin D, NNS; number needed to screen, GDM; gestational diabetes mellitus

baseline and at delivery, are depicted in Fig. 2 and Supplemental Fig. 1 and their equivalent Supplemental Tables 1 and 2, respectively.

Table 4 compares the OR of various protocols to achieve 25(OH)D levels of >20 ng/mL at delivery; protocol I4, compared with other protocols, obtained the optimum result for moderately deficient cases (OR, 1.7; 95% CI, 1.2 to 2.4), indicating that by using this protocol for moderate deficiency, the odds of achieving 25(OH)D levels of >20 ng/mL at delivery was 1.7-fold higher than for the no intervention group. In the severe group, protocol I6 indicated the best results (OR, 2.3; 95% CI, 1.7 to 3.3). Differences in in maternal 25(OH)D levels based on the type of supplementation regimen in the screening site indicated that per increase in supplementation dosage, maternal 25(OH)D levels increased as well (Supplemental Fig. 2).

Discussion

To the best of our knowledge, this is the largest prospective study to date exploring the effectiveness of antenatal vitamin D screening vs nonscreening on maternal

Table 3. Achievement of 25(OH)D >20 ng/mL Levels at Delivery Based on Screening Sites

Outcomes	25(OH)D at baseline	N(%)[@] Screening site (Masjed-Soleyman)	N(%)[@@] Non-screening site (Shushtar)	AEPN(%) (95% Cl) NNS (95%CI)	AEPN (%) (95% Cl)
Achieving 25(OH)D> 20ng/ml at delivery	**Moderate deficiency**	197(0·50)	11(0·03)	0·47 (0·42-0·52) 2(2-3)	
	Severe deficiency	218(0·55)	0(0·00)	0·55 (0·50-0·61) 2(1-2)	
	***Total**	415(0·53)	11(0·02)	0·51 (0·48-0·55) 2(1-3)	0·4 0·5 0·6

*Total=Moderate +Severe vitamin D deficiency cases at baseline; [@]No. cases in screening site (%); [@@]No. cases in non-screening site (%),
25(OH)D; 25-hydroxyvitamin D, AEPN; Absolute Excess Probability of Becoming Normal vitamin D case (95%CI), NNS; number needed to screen

outcomes, followed by comparison of different supplementation approaches based on the initial extent of deficiency. The relationship between 25(OH)D concentration and maternal outcomes has so far been investigated in a number of clinical trials; however, as these studies were not primarily designed to evaluate vitamin D deficiency through a screening program, no universal screening has yet been recommended.

We showed that implementing a screening program for detection and treatment of maternal vitamin D deficiency can effectively reduce maternal outcomes, including preeclampsia, GDM, and preterm delivery. In the screening arm, those women who were taking a monthly maintenance dose of 50,000 IU of D3 had a higher probability for achieving serum 25(OH)D >20 ng/mL; this probability in the intervention and nonintervention sites was 53% and 0·02%, respectively, indicating that without a screening program, 98% of unscreened women (either with moderate or severe deficiency) remained deficient at delivery, which is a considerable number. Specifically, these values in the moderately and severely deficient cases were 50%, 0.03%, and 55%, 0%, respectively, which indicates a slightly high significance of screening among severely deficient women compared with that of their moderately deficient counterparts. In accordance with previous findings, our intervention was more efficient among severely deficient cases, which supports the notion that the response to supplementation is directly affected by baseline 25(OH)D concentrations, as severely deficient patients seem to benefit more from supplementation (12, 13).

Our findings with the current study design and subgroup classifications indicated a dose-response increase. However, there is the likelihood of producing comparable results had we used the same (lower dose) regimen for all deficient women (irrespective of their extent of deficiency), because evidence confirms that in subjects with a minimum 25(OH)D concentration of 20 ng/mL, levels can increase up to 1 ng/mL for every 100 IU of vitamin D, regardless of low- or high-dose supplementation (14, 15). However, a meta-analysis explored the influence of vitamin D supplementation on serum 25(OH)D concentration based on 76 trials and reported no statistically significant association between high 25(OH)D concentration at baseline and lower increases in 25(OH)D concentrations (16).

We found that screening could overall reduce risk of complications (preeclampsia, GDM, and preterm delivery) by 55%; following screening, only 17% of women progressed to adverse outcomes, whereas without screening 29% of pregnancies were complicated with either one or more of these outcomes. Without screening, 17% of women developed preeclampsia, compared with 8% among screened women, indicating a high rate of preeclampsia even among our low-risk pregnant population. This is of course comparable to the high prevalence of the condition in some other developing countries (17) and might somehow indicate the underlying risk factors

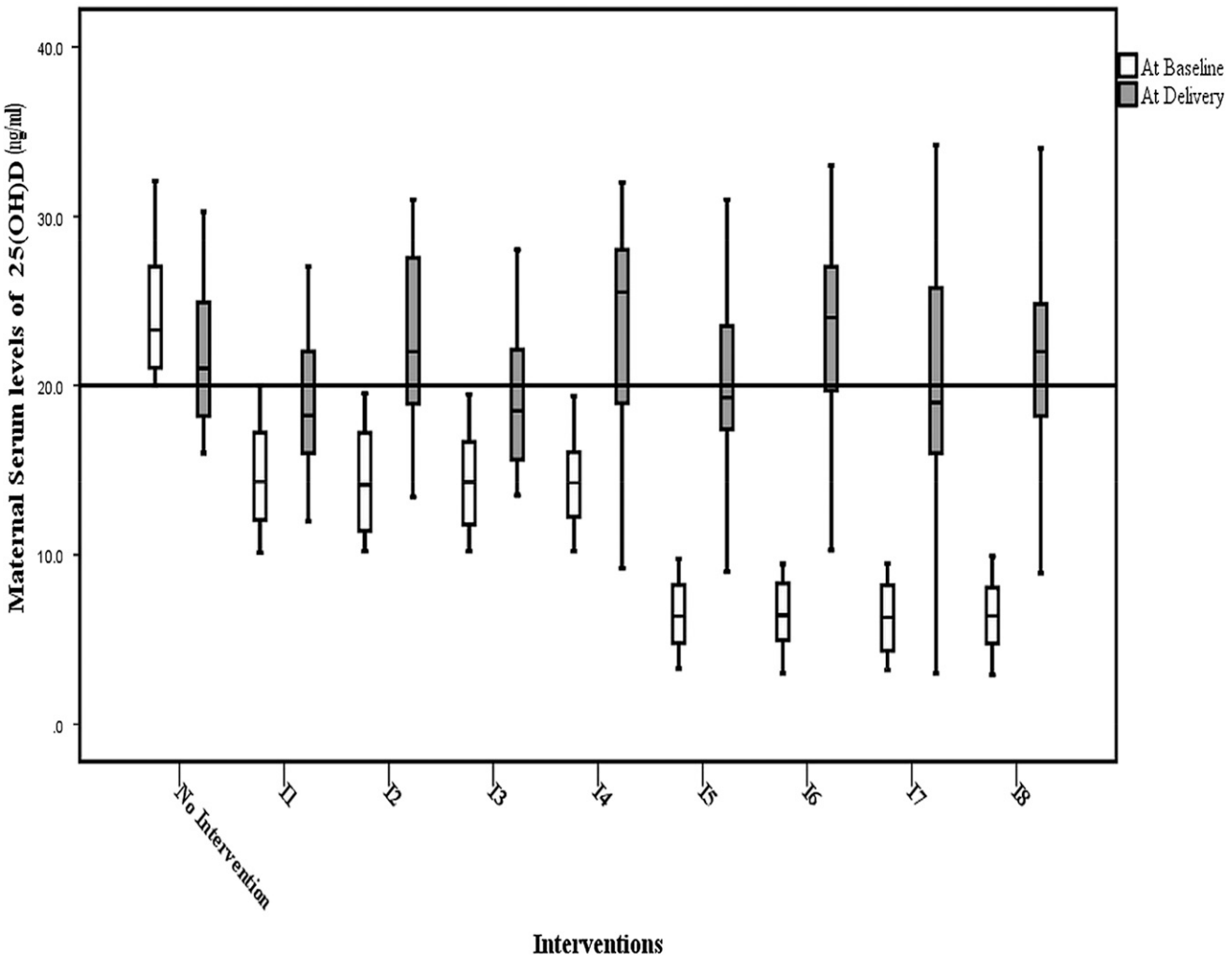


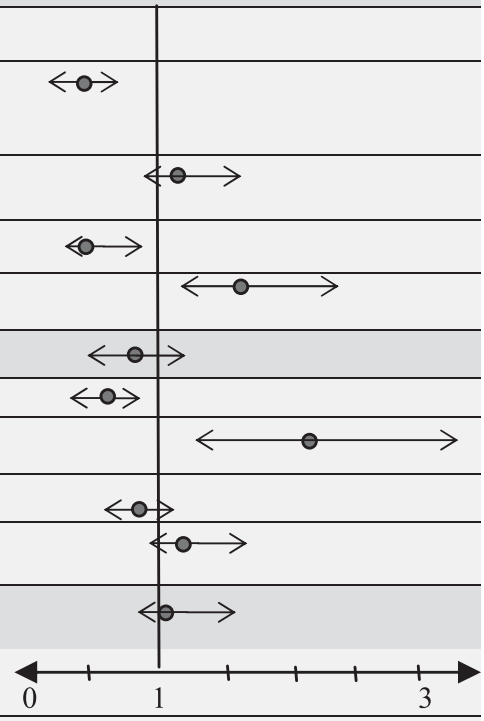
Figure 2. Maternal 25(OH)D levels by type of intervention in the screening site (Masjed-Soleyman) at baseline and at delivery (I1: 50,000 IU weekly for 6 wk; I2: 50,000 IU weekly for 6 wk plus monthly maintenance dose of 50,000 IU; I3: a single dose of 300,000 IU; I4: a single dose of 300,000 IU plus monthly maintenance dose of 50,000 IU; I5: 50,000 IU weekly for 12 wk; I6: 50,000 IU weekly for 12 wk plus monthly maintenance dose of 50,000 IU; I7: 300,000 IU, two doses for 6 wk; I8: 300,000 IU, two doses for 6 wk plus monthly maintenance dose of 50,000 IU).

such as the extremely low levels of 25(OH)D at baseline and at delivery. Importantly, remember that there is not a present therapy to prevent or treat preeclampsia and but a single treatment to prevent preterm birth by injecting 17α-hydroxyprogesterone, which is expensive and appears to not work (18). Women who have preeclampsia have lower concentrations of 25(OH)D compared with normotensive ones (19); reports from a meta-analysis including seven observational studies revealed that risk of preeclampsia is 79% higher in women who are deficient in vitamin D [OR, 1.79 (1.25 to 2.58)] (2). Another recent meta-analysis of RCTs found that vitamin D supplementation during pregnancy was of little value with respect to complications of birth (6). However, that analysis was deeply flawed in that most of those RCTs administered so little vitamin D that genomic alteration would not have occurred, yet alone induced biologic function (20). Our findings of a 9% decrease in preeclampsia risk in supplemented women is comparable to that of a recent Cochrane Review suggesting a 6.6% lower risk of preeclampsia in women receiving supplementation compared with those with no intervention or placebo [8.9% vs 15.5%; risk ratio, 0.52% (0.25% to 1.05%) (5).

Several biologic mechanisms are involved in the inverse association between vitamin D and preeclampsia. Vitamin D is a modulator of proinflammatory responses and is therefore able to reduce oxidative stress and promote angiogenesis through vascular endothelial growth factor and gene modulation (21); it can also lower blood pressure through suppression of the renin–angiotensin system (22). Furthermore, vitamin D has been shown to be essential for normal vascular development as the embryo implants and develops in the uterus (23). As such, it has been proposed that for preventing an outcome such as preeclampsia, the pathophysiological process of which initiates in early pregnancy, the most appropriate time for supplementation would be from the first trimester (24). In

Table 4. Achievement of 25(OH)D >20 ng/mL Levels at Delivery Per Protocol in Interventional Site

Outcome	Intervention	N(%) Screening site (Masjed-Soleyman)	OR(95% CI)	OR (95% Cl)
Achieving 25(OH)D> 20ng/ml at delivery	I_0	53(54)	----	
	I_1	34(35)	.5(.3-.6)	
	I_2	58(59)	1·2(·9-1·7)	
	I_3	38(39)	·5(·4-·8)	
	I_4	67(67)	1·7(1·2-2·4)	
	Sub-Total	197(0·50)	0·8(0·5-1·3)	
	I_5	40(41)	0·6(·4-·8)	
	I_6	72(73)	2·3(1·7-3·3)	
	I_7	47(47)	0·8(0·6-1·1)	
	I_8	59(60)	1·3(0·9-1·7)	
	Sub-Total	218 (0·55)	1·1(0·8-1·6)	



25(OH)D; 25-hydroxyvitamin D

I_0: No intervention (reference group: 25(OH)D levels of > 20ng/ml)

I_1: 50,000IU oral D3 weekly for 6 weeks

I_2: 50,000IU oral D3 weekly for 6 weeks + monthly maintenance dose of 50,000IU until delivery

I_3: A single dose of intramuscular 300,000IU D3

I_4: A single dose of intramuscular 300,000IU D3+monthly maintenance dose of 50,000IU until delivery

I_5: 50,000IU of oral D3 weekly for 12 weeks

I_6: 50,000IU of oral D3 weekly for 12 weeks + monthly maintenance dose of 50,000IU until delivery

I_7: Intramuscular 300,000IU D3; 2 doses for 6 weeks

I_8: Intramuscular 300,000IU D3; 2 doses for 6 weeks+ monthly maintenance dose of 50,000IU until delivery

this context, different timings of interventions may explain the discrepancy between the results. The neutral effect of supplementation is more probable in studies that put women on supplementation at ≥20 weeks of gestation, because they might have missed the regulatory effect of vitamin D on placentation in early pregnancy (21).

The Vitamin D Antenatal Asthma Reduction Trial found no effect of supplementation on reducing preeclampsia [8.08% vs 8.33%; relative risk, 0.97 (0.61 to 1.53] when analyzed in an intent-to-treat format (25). However, when the Vitamin D Antenatal Asthma Reduction Trial data were analyzed taking into account first trimester 25(OH)D levels, preeclampsia rates were significantly reduced (25, 26).

We also found that our intervention helps in decreasing GDM by 50% in women with 25(OH)D <20 ng/mL. Despite very-low-quality evidence regarding the supplementation effect on GDM, which have led to inconclusive data (5), some studies have documented a causal association between serum 25(OH)D levels and impaired glucose metabolism (27, 28). Also note that besides the negative effect of vitamin D deficiency on β cell function (29), some novel evidence recently revealed a complex interplay between 25(OH)D, glucose homeostasis, and PTH, demonstrating that only elevated PTH with simultaneous low levels of 25(OH)D can contribute to insulin resistance (30). This phenomenon may partly justify the inconsistency between results, although further investigations are warranted.

We also found that supplementation could decrease the risk of preterm delivery up to 40% [0.6 (0.4 to 0.8)], findings in agreement with earlier reports on an inverse association between maternal 25(OH)D and preterm delivery (31, 32). Because vitamin D provides innate immune and antibacterial responses in placental cells, it is assumed that vitamin D reduces risk of preterm delivery through anti-inflammatory mechanisms (33). However, combined supplementation of vitamin D plus calcium may have led to null or even converse results in this respect (3).

Apparently conflicting results in trials are not unexpected; studies are apparently heterogeneous in terms of sample size, timing, and duration of intervention, comparison groups (no intervention, placebo, calcium, or other mineral supplements), types of vitamin D (D_2 or D_3), frequency of supplementation (continued or single dose), as well as other influencing factors (primary endpoints, latitude, veiling styles, skin pigmentation, ethnicity, seasonality, body mass index, and smoking).

Another debatable issue is the definition of optimal serum 25(OH)D levels and consequently the precise daily dose required for individuals. Several studies have so far supported the effects of high-dose supplementation on optimization of 25(OH)D levels during pregnancy (34–36). A double-blind controlled trial, comparing different D3 doses of 400, 2000, and 4000 IU/d, reported that mothers who were randomized to 4000 IU yielded the best results in achieving sufficiency (≥32 ng/mL) at term and 1 month prior to delivery (serum level at delivery 46 vs 23 ng/mL at baseline) (35). This same study was the first to report that vitamin D supplemented at a high level during pregnancy could decrease birth complications (37). This study demonstrated that as vitamin D supplementation increased from 400 to 4000 IU/d, birth complications decreased. These results also argue for the highest vitamin D repletion treatment regimen presented in the current study. Reports from another placebo-controlled trial showed that weekly doses of 35,000 IU of D3 in the third trimester could significantly raise maternal 25(OH)D levels at delivery compared with the placebo group, with a mean difference of 38 ng/mL (36).

Evolving evidence now recommends a minimum daily intake of 4000 IU preconceptionally and in early pregnancy to maintain 25(OH)D levels at least >40 ng/mL (26, 38). This recommendation is put forth for general populations of pregnant women and, of course, to achieve the optimal level in women who have hypovitaminosis D, doses >4000 IU/d would be required.

Similar to earlier reports, our finding of ~7000 IU/d of D3 within 6 to 12 weeks in women with moderate to severe deficiency endorses the need for higher prenatal dosing, particularly among vitamin D–depleted populations. The present findings, carried out in a low latitude mostly sunny province, indirectly imply a larger proportion of at-risk women living at higher latitudes of Iran. Besides, factors such as limited access to fortified products, covered clothing, and dark skin types can further exacerbate the problem.

With respect to safety concerns, humans are generally able to produce much higher doses of up to 25,000 IU/d when total body exposure is practiced in intense sunlight or for at least 20 minutes (39). Vitamin D intoxication is extremely rare and typically progresses after long-term consumption of >10,000-IU doses, with serum concentrations of >150 ng/mL (40). The Institute of Medicine determined an upper tolerable intake level of 4000 IU/d for vitamin D in pregnancy, whereas the Endocrine Society suggests an upper limit of 10,000 IU. Because, in our trial, maternal serum levels of 25(OH)D in the supplemented group barely reached 20 ng/mL at delivery, the possibility of toxic effects and hypercalcemia would be minimal. Moreover, no adverse outcome or complaints of clinical features attributable to supplementation were reported by any of our study subjects. It is arguable that our supplementation program could even be more aggressive to achieve higher circulating levels of 25(OH)D and decrease birth complications further.

The present data have an especially important meaning to the African American population in the United States who present with the same low 25(OH)D levels during pregnancy (35). African American women have a much higher rate of pregnancy complications as compared with the white population (41). Many factors have been implicated in the bias; however, vitamin D deficiency is not one of them (42). The obstetrics community in the United States and elsewhere think that the 400 IU/d vitamin D recommended by the Institute of Medicine is adequate (7). This assumption has been demonstrated to be inaccurate by a recent meta-analysis of low vitamin D intakes to prevent/treat complications of pregnancy (6). The data from our study should go a long way to modernize the true requirement for vitamin D during pregnancy.

The major strength of the study lies in the subject recruitment; that is, a large number of participants stratified based on the initial 25(OH)D levels were supplemented using different D3 supplementation approaches. With a compliance rate of 98.5%, the present findings have the generalizability required for the population level. Our findings are subject to certain limitations. First, because the intervention was performed from 14 weeks of gestation onward, we had no evidence regarding its efficacy during early pregnancy loss and, more importantly, on its safety in the entirety of pregnancy. Second, as data collection was carried out throughout the year, we were unable to adjust for seasonal variance; however, the impact of seasonality on UV radiation appears to be the greatest at high latitudes having low sun exposure in winter than at lower latitudes (43). Third, we were not able to recruit liquid chromatography technique to quantify 25(OH)D values; however, the ELISA technique is considered as a reliable method when performed by experienced staff (44). Alternatively, typically, imprecisions of ELISA measurement occur at high levels of 25(OH)D, and in the case of any inaccuracy in our measurements, it could have been applied equally to both our screening and nonscreening sites and, hence, the overall conclusions of our study might not have been influenced. Finally, owing to the diversity of food supplies, we had no information on vitamin D dietary intakes in our participants.

To conclude, the screening and supplementation policy could effectively detect and treat women who were deficient in vitamin D and improve adverse outcomes compared with the nonscreening site. Moreover, in the absence of any untoward supplement-related outcome, we cautiously conclude that doses of 300,000 IU in moderately deficient women and 600,000 IU (divided in two doses) in severely deficient women, followed by a monthly maintenance therapy, could be well tolerated with a high rate of patient compliance to treatment.

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