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Randomized Control Trials

Effect of vitamin D3 supplementation in pregnancy on risk of pre-eclampsia – Randomized controlled trial

Aisha Mansoor Ali, Abdulaziz Alobaid, Tasnim Nidal Malhis, Ahmad Fawzi Khattab*

Women Specialized Hospital, King Fahad Medical City, P.O. Box 59046, Riyadh, 11525, Saudi Arabia

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SUMMARY

Background: Vitamin D plays pivotal role in decidualization and implantation of the placenta. Recent researches have shown that low level of vitamin D3 "25-hydroxyvitamin D (25[OH]D)" in serum is a risk factor for pre-eclampsia. Latest evidence supports role of vitamin D3 deficiency treatment in reducing the risk of pre-eclampsia. The aim of this study is to determine the effect of antenatal supplementation of vitamin D3 on the risk of pre-eclampsia and to explore the dose effect in attaining the vitamin D3 normal level.

Method: An open labelled randomized controlled study was conducted on 179 pregnant women presenting in King Fahad Medical City antenatal clinic from Oct 2012–Oct 2015. Patients with age less than 20 years or more than 40 years, pregnancy with fetal anomalies, history of hypertension, pre-eclampsia, recurrent miscarriage, chronic renal or hepatic disease and malignancy were excluded from the study. Serum 25[OH]D was analysed during the first trimester (between 6 and 12 weeks of pregnancy). Patients with vitamin D3 deficiency (serum levels <25 nmol/L) were included in the study and randomized for vitamin D3 supplementation 400 IU (Group 1) versus 4000 IU (Group 2). Both groups were compared for the prevalence of pre-eclampsia and dose effect on vitamin D level.

Results: Of 179 gravidae enrolled, 164 completed the trial. Mean maternal 25[OH]D was significantly increased in group 2 from 16.3 ± 5 nmol/mL to 72.3 ± 30.9 nmol/mL compared with group 1 from 17.5 ± 6.7 nmol/mL to 35.3 ± 20.7 nmol/mL ($p > 0.0001$). The relative risk reduction (RRR) for attaining ≥75 nmol/L before delivery was significantly higher (RRR 93.2 [CI 79–98] when treated with 4000 IU. The total incidence of pre-eclampsia in the study population was 4.3%. In comparison to group 1, the group 2 reported fewer pre-eclampsia events during the study period (8.6% versus 1.2%; $p < 0.05$). The total number of IUGRs was lesser in the group 2 (9.6%) versus group 1 (22.2%); $p = 0.027$. However, other obstetric outcomes were comparable between both groups.

Conclusion: Vitamin D supplementation in the deficient group reduces the risk of pre-eclampsia and IUGR in a dose dependant manner. However larger clinical trials are essential to investigate optimum dosage of vitamin D3 in this group.

1. Introduction

Vitamin D deficiency has potential implications on maternal health, and the offspring [1,2]. A multi-ethnic study conducted in London antenatal population exhibited that Middle Easterns (64%) were highest in number to have vitamin D deficiency (level <25 nmol/L) as compared to Asian Indians, black women and Caucasian [1]. Vitamin D stimulates immune reaction in decidual tissues and may help in healthy placental function [2,3]. Research suggests the imbalance levels of placental biomarkers and vitamin D deficit as risk factors for the occurrence of preeclampsia [4,5].

Pre-eclampsia is a multifactorial disorder that ensues beyond twenty weeks of gestation [6]. Globally, pre-eclampsia carries substantial risks to mother and fetus [7,8]. Based on the higher incidence of pre-eclampsia in the underdeveloped world and its leading health disasters, World Health Organization recommends to perform research work aiming to lessen the maternal and child health risks (2.8% versus 0.4% of live births respectively) [9].

* Corresponding author.
E-mail address: akhattab@kfmc.med.sa (A.F. Khattab).

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Many studies indicate positive association between vitamin D deficiency and pre-eclampsia [10–15]. However, Cochrane review 2012 regarding the association of hypovitaminosis D in pregnancy reported inconclusive evidence for pre-eclampsia and other pregnancy outcomes [16]. As expectant mothers are highly prone to nutritional imbalance specially vitamin D, they must be supplemented to avoid potential risks to mother and fetus [17]. Recent literature has alarmed the rising prevalence of Vitamin D deficiency worldwide [18] and in Saudi Arabia [19].

Literature established inverse association of maternal delivery 25[OH]D levels and comorbidities of pregnancy [20,21] which include pre-eclampsia [22,23], gestational diabetes, an increased rate of caesarean section [23,24], low birth weight [10,25] and preterm delivery [26]. However, other studies negate this finding [27–31].

The routine daily antenatal vitamin D is 400 IU while safest maximum recommended dose for vitamin D supplementation in pregnancy is 4000 IU [20,28]. Vitamin D is a likely contender for pre-eclampsia, and recent systematic reviews were suggestive of its protective association, advocating randomized controlled trials (RCTs) to test its effectiveness and safety [32].

Given the high prevalence of a vitamin D deficiency in Saudi population [33,34] and increased number of preeclampsia cases in Saudi women [35], the present was attempted to analyse and add the better understanding of the role vitamin D supplementation in reducing the risk of pre-eclampsia.

Many studies concluded the need for high-quality randomized trials to know the recommended dose of vitamin D supplementation in pregnancy [16,36]. A recent paper published voices to conduct trials on selected population to conclude, under which circumstances vitamin D supplementation would be beneficial [37]. An Indian RCT published in 2015 also proved favourable impact on maternal and offspring outcome upon vitamin D replacement in a pregnant population. In the same way a Pakistani study concluded the same [38]. Though there is a consistency of evidence published regarding achieving optimized maternal and neonatal vitamin D status by antenatal supplementation with higher doses of up to 4000 IU [39] yet Randomized Controlled Trials (RCTs) with conflicting results for obstetric outcomes and heterogeneity in study design calls for uniformity in methodology for future research [39,40].

We postulated that high dose (4000 IU) vitamin D supplementation in vitamin D deficient pregnant population would reduce the risk of pre-eclampsia and helps to attain the vitamin D3 normal level.

2. Research methodology

2.1. Study design

The study has two parallel groups, and it is an open labelled randomized controlled trial. It was approved by The Institutional ethical committee, The Institutional Review Board for Human Research (# H-01-R-012), OHRP/NIH, USA, The Institutional Review Board for Human Research (#00008644) and registered in ClinicalTrials.gov Identifier: NCT03101150.

2.2. Study population and setting

The study was conducted from October 2012 through October 31, 2014 at King Fahad Medical City antenatal clinic. The inclusion criteria for the subjects included maternal age of 20–40 years, confirmed singleton pregnancy of less than 13 completed weeks of gestation at the time of consent, planned to receive ongoing prenatal care in King Fahad Medical City antenatal clinic, the ability to provide written informed consent at the first visit, low risk pregnancy and 25[OH]D levels less than <25 nmol/L.

2.3. Exclusion criteria for the study

Mothers with abnormal foetus, history of hypertension, pre-eclampsia, recurrent miscarriages, chronic kidney disease, chronic liver disease, and malignancy were excluded from the study.

2.4. Gestational age at enrolment

Subjects were assessed for eligibility. The eligible participants were consented and enrolled into the study at 6–12 weeks of gestation. Gestational age was based on last menstrual period. In case of unsure or unknown last menstrual period, gestational age was calculated by fetal scan, and obstetrical estimate of expected date of delivery was recorded. All cases were followed till 3 months postpartum.

2.5. Initial study visit

All participants completed questionnaires which included information regarding socio-demographic information, baseline health status and medical history at the first visit. Baseline blood and urine samples were obtained following each participant's consent at the initial visit (10 to <13 weeks). Irrespective of enrolment gestational age, vitamin D supplementation did not begin before the twelfth week of gestation (12 weeks and six days).

2.6. Randomization

Eligible and consented study subjects were randomized according to permuted block design scheme to be allocated in group 1 (G1) or group 2 (G2) generated by biostatistician. The subjects were enrolled by co-investigators and allocated to intervention by primary investigator.

2.7. Intervention

G1 at the time of each subject's enrolment was prescribed “Materna” Multivitamin-Multimineral Supplement (distributed by Wyeth) containing 400 IU vitamin D3/tablet once daily. G2 was prescribed 4000 IU vitamin D3 (40 drops daily) “Vidrop” by Medical Union Pharmaceuticals (MUP). Intervention was commenced from 13 weeks gestational age.

2.8. Outcomes

Primary Outcome Measure(s):

1. Number of participants with pre-eclampsia in both groups [Time Frame: From 20 weeks of pregnancy till event of pre-eclampsia seen, whichever came first, assessed up to 32 weeks].

Secondary Outcome Measure(s):

2. Change in vitamin D level at 36th week of pregnancy.
3. Number of patients with Intrauterine Growth Retardation (IUGR) at delivery.

2.9. Drug accountability

Both treatments were dispensed by KFMC pharmacy to respective randomized patients and supply was given enough till next visit. Patients were instructed to return unused medications. At the end of study, only three patients returned less than 10 tablets in group 1 and 5 patients returned full to half-filled single bottles in group 2. Vitamin D status of these 5 patients at the end of the study was improved to insufficient level.

2.10. Subsequent study visits and data collection

Participants were followed with monthly study visits, which continued until twelve weeks postpartum. These visits coincided with routine obstetrical visits. A case report form was completed for each patient including the following patient information: personal data (maternal age, parity, BMI), fetal scans, Oral Glucose Tolerance Test (OGTT) at 24–28 weeks, serum vitamin D3 level pre and post supplementation. For monitoring of adverse effects, parathyroid hormone, serum calcium, liver and renal function tests were performed. Data were maintained in MS Access database, and excel spread sheets. Determination of gestational age by ultrasound, blood pressure, weight gain, proteinuria and growth scans were done 4–6 weekly. Each case was followed up until 12 weeks postpartum to access event of pre-eclampsia. Pre-eclampsia was defined as the new onset of hypertension and proteinuria after 20 weeks of gestation. Hypertension was defined as systolic blood pressure greater or equal to 140 mmHg and/or diastolic pressure greater than or equal to 90 mmHg, and/or an increase of greater than 30 systolic and/or an increase of greater than 15 diastolic. Proteinuria was defined as urine protein greater than 300 mg/24 hr or 2 + dipstick [6].

2.11. Laboratory measurements

The 25[OH]D levels were measured using the Roche Diagnostic Vitamin D total assay, competitive electro-chemiluminescence-protein binding assay intended for the quantitative determination of total 25[OH]D in human serum and plasma. This assay employs the vitamin D binding protein (VDBP) as a capture protein that binds to both 25[OH]D3 and 25[OH]D2 (Roche Diagnostics, Mannheim, Germany). Between day precision was coefficient of variance = 4.9% and 1.9% at mean concentration of 43.3 and 105 nmol/L respectively using quality control material provided by Roche Diagnostics.

Vitamin D status was classified as follows: Deficiency: <25 nmol/L, Insufficiency: 25–75 nmol/L, Sufficiency: 75–200 nmol/L and Toxicity: >250 nmol/L [41,42].

3. Statistical methods

3.1. Sample size and power considerations

A sample size of minimum 152 was calculated to be able to determine incidence of pre-eclampsia that is in the range of 8–17% with 80% power assuming an alpha of 5%.

3.2. Statistical analysis

Continuous demographic and clinical data was summarized by means and standard deviations. Categorical data was analysed by frequencies and percentages. Statistical comparisons of the incidence of pre-eclampsia between the two study groups was based on chi-square. Multivariate analysis was carried out using logistic

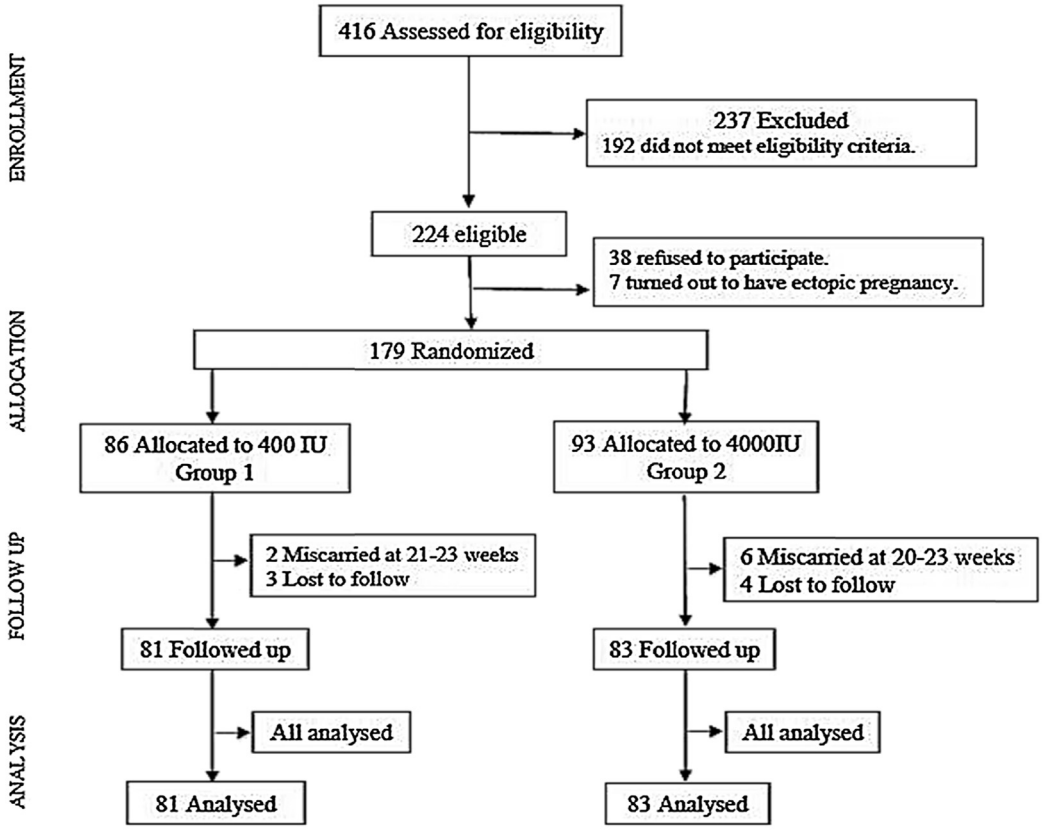

Fig. 1. Flow chart of trial.

regression principles. Multiple comparisons were controlled using Bonferroni criteria and an overall 5% significant level. The analysis was carried out using SPSS version 22.

Because the primary outcome was pre-eclampsia from 20 weeks to 12 weeks after delivery, the primary analysis was restricted to participants who remained in the study until delivery and provided a blood sample within 12 weeks before delivery (completers-only analysis).

4. Results

4.1. Study population

Figure 1 shows the enrolment, allocation, and follow-up of the women who participated in the trial. Of 179 gravidae enrolled, 164 completed the trial. Table 1 shows the demographic features of the study population. The comparison between the two study groups did not show statistical difference.

Table 1
Demographic of patients.

Demographics	Vitamin D supplementation		*p* value
	400IU(n = 81)	4000IU(n = 83)	
	Mean (±SD) N [%]		
Age	29.3 (5.3)	29.4 (4.8)	0.899
BMI	28.8 (7.2)	28.1 (7.3)	0.537
Saudi nationality	80 [98]	80 [96]	0.1
Primiparity	31 [38]	33 [40]	0.5

* Significant (if p-value ≤ 0.05), Standard Deviation (±SD).

4.2. Primary outcome

The total incidence of pre-eclampsia in the study population was 4.3%, G2 had significantly lower incidence than G1 (p = 0.049). Comparison of the two groups showed that the group supplemented with vitamin D 4000 IU reported significantly fewer pre-eclampsia events during the study period than patients receiving 400 IU vitamin D, (1.2%; vs. 8.6% p < 0.05) see Table 2. However, the only patient who developed preeclampsia in G2 had vitamin D levels reaching normal by delivery. Comparison between the levels of vitamin D attained after treatment considering the different study characteristics showed no significant difference between the two study groups (Table 2). Table 4 depicts that pre-eclampsia risk reduces by 16.3% in intervention group with 95% CI (0.02–1.32).

Table 2
Maternal and fetal outcomes.

	Vitamin D supplementation		*p* value
	400 IU(n = 81)	4000 IU(n = 83)	
	N (%)		
Caesarean delivery	28 (34)	28 (33.7)	0.860
Preterm delivery	13 (15)	13 (14)	0.498
Miscarriage	2 (2.3)	6 (6.5)	0.166
IUGR	18 (22.2)	8 (9.6)	0.027*
Gestational DM	9 (10.8)	7 (8.6)	0.635
Pre-eclampsia	6 (7.4)	1 (1.2)	0.049*
Gestational age at delivery	38.2 (2.2)	38.0 (2.0)	0.543
Birth weight	2.94 (0.5)	2.89 (0.5)	0.522

* Significant (if p-value ≤ 0.05).

4.3. Secondary outcomes

A significant improvement in maternal 25[OH]D was noticed in G2 (16.3 ± 5 nmol/mL vs. 72.3 ± 30.9 nmol/mL) as compared to G1 (17.5 ± 6.7 nmol/mL vs 35.3 ± 20.7 nmol/mL) (p > 0.0001) as shown in Table 3. The relative risk reduction (RRR) for achieving vitamin D sufficiency before delivery was significantly high (RRR 93.2 [CI 79–98]) when treated with 4000 IU. Vitamin D sufficiency was achieved in 48.9% versus 3.5% patients in G2 as compared to G1 (p < 0.0001). We observed in 4000 IU dose group that 6.5% retained the deficiency status, while remaining 44.6% antenatal ladies had an upgrade of insufficiency level from deficiency status. In G1 majority (62.8%) were deficient status before delivery. However 33.7% improved to insufficient level of vitamin D (see Figure 2).

Table 3
Maternal vitamin D serum levels.

Vitamin D serum level	Vitamin D supplementation		*p* value
	400 IU (n = 81)	4000IU(n = 83)	
	Mean (±SD)		
Pre-supplementation	17.5 (6.7)	16.3 (5.0)	0.196
Post supplementation	35.3 (20.7)	72.3 (30.9)	0.0001*

* Significant (if p-value ≤ 0.05), Standard Deviation (±SD).

The total number of IUGRs in G2 was 8 (9.6%) versus 18 (22.2%) in G1 (p < 0.0125). Risk of IUGR significantly falls by 43% in intervention group (Table 4).

5. Discussion

The results of this study showed a total incidence of pre-eclampsia in vitamin D deficient pregnant ladies was 4.3%. Treatment with the dose of 4000 IU led to attain sufficient level in almost 49% with RRR (93.2) for achieving sufficiency. Moreover, the dose of 4000 IU significantly had the lower incidence of pre-eclampsia and IUGR compared to the lower dose which represents the routine supplementation. The results highlighted the association of vitamin D levels and the incidence of IUGR and pre-eclampsia. Moreover, it reflected the dose effect on improving the vitamin D level during pregnancy.

Pre-eclampsia incidence reported in the current study is comparable to earlier reports [9]. We found a higher incidence of pre-eclampsia in the routine dose group. Our study population is comprised of the vitamin D deficient group (serum levels <25 nmol/L). The current study tested the hypothesis that vitamin D supplementation could decrease the number of pre-eclampsia cases in dose dependent manner, and we found a favourable results of vitamin D supplementation in both high and normal dose, although the 4000 IU showed a major effect in reaching the normal level. Moreover, we started supplementation early in the second trimester thus strengthening an inverse relationship between 25-OH vitamin D levels and pre-eclampsia [43–46]. We selected this group based on the evidence that they are at higher risk of developing pre-eclampsia [22,23,47]. Zabul et al. concluded that the suboptimal production of vitamin D3 derivatives possibly damage oxidative stress processes on-going in placental tissue and escalate the risk of pre-eclampsia. Therefore, they suggested that supplementation with high dose vitamin D3 might be a logical, safe and reliable remedy [48]. Furthermore, it has been presumed that vitamin D3 plugs off inflammatory pathways and hinders endothelial cell damage [49]. Vitamin D not only works through inflammatory processes

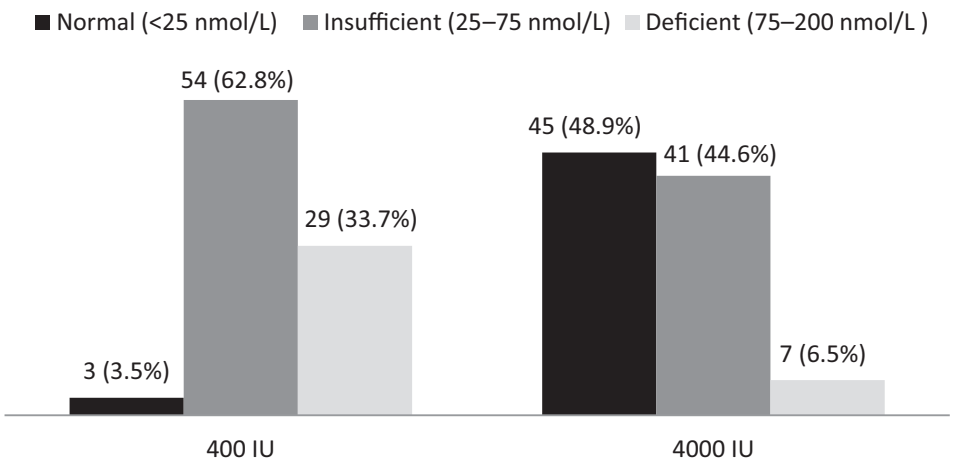

Fig. 2. Vitamin D status post intervention before delivery (30–36 weeks of gestation).

Table 4
Risk estimation of pre-eclampsia and IUGR.

Risk estimation	Relative risk	95%CI	NNT
Pre-eclampsia	0.163	(0.02–1.32)	16
IUGR	0.43	(0.19–0.94)	79

Confidence Interval (CI), Number needed to treat (NNT).

regulation but also modifies placental vasculature by its metabolic and endocrine role [50,51].

In contrast to our results, few studies have not commended the positive association between vitamin D and pre-eclampsia. These studies reported similar levels of vitamin D in pre-eclampsia or eclampsia patients compared to controls [30,52]. Unfortunately, they measured the levels of vitamin D in early gestation, and it is known that some changes occur in early pregnancy including increased maternal serum calcitriol, placental vitamin D receptors and renal and placental CYP27B1 cytochromes activity, without changes in serum 25[OH]D or calcium levels [51]. The cortisol levels could have negative feedback on vitamin D levels. Moreover, their study designs were either case control with confounders or RCT with different suboptimal doses.

Many recent studies negate the association between vitamin D low serum levels and the development of pre-eclampsia, gestational hypertension or preterm birth [27–31]. This can possibly be elucidated by the great heterogeneity in some RCTs performed on this issue. For example, they vary in ethnical, environmental (healthy/disease), topographical (northern/southern), baseline vitamin D levels, dose and regime of vitamin D (400 IU/2000 IU/4000 IU) and drug initiation time. However, there are no clinical trials conducted with early intervention in the vitamin D deficient group of the pregnant population to establish an association with pre-eclampsia. In this study, we managed with early treatment to show high RRR in both doses used. Our results are augmenting the reports by Hollis et al. who promoted the daily antenatal dose of 4000 IU/day in achieving sufficiency [20]. Similar results with early intervention were achieved previously even with a dose of 2000 IU [49].

Our research subjects belong to a deficient group of vitamin D in both arms of the trial, and we supplemented the patients with maximum safest dose in the intervention arm. The limitation of the study was blinding to treatment. Since vitamin D is fat soluble and its dilution requires alcohol which was an ethical issue for our study population, we treated the control group with multivitamin tablet instead of diluted drops of 400 IU vitamin D. We observed that even with 4000 IU dose only half of the group achieved the normal level and among the other half 6.5% retained the deficiency status. With the reporting of high drug accountability in our study, this effect could be due to intolerance of medication. It is also important to mention that adverse events in both study groups were not analysed. Therefore, trials with higher doses under meticulous supervision can be done. In line with our finding a report from Poland showed that supplementation of 800–1000 IU daily was not enough to attain the sufficiency level [53].

Vitamin D supplementation especially with a dose of 4000 IU resulted in significant lessening in the incidence of IUGR, providing bonus role of vitamin D in improving pregnancy outcomes. Our results support previous reports that showed antenatal vitamin D status in the mid gestation was associated with risk of small for gestational age babies, and even it has some fetal gender effect [9,54,55]. However, our study is not in agreement with many cohort studies from different countries that showed no influence of vitamin D levels on neonatal outcomes. We would like to highlight the possibility of better vitamin D status in their study population or that they used higher cut-offs (37.5–80 nmol/L) for vitamin D deficiency [56–60].

The strength of this study is randomized clinical trial study design. Furthermore, the findings of our study are generalizable to other Kingdom of Saudi Arabia as our institution is a referral institute and patient were referred from all over the Kingdom. However, the current study can be interpreted with the certain limitation that it is an open label, lacks naive or placebo control group as well as it did not document the laboratory finding of metabolic indicators for dose effects. Hence, modern RCTs should take into account inclusion of multi-ethnic participants residing in moderate climate where access to sunlight and thus vitamin D3 synthesis is lesser. Moreover, a double blind study would confirm the findings. Researchers are advocated to initiate similar studies on women with prior pre-eclampsia event to assess the risk of recurrence.

6. Conclusion

Our study concluded that 4000 IU daily dose of vitamin D reduces the pre-eclampsia risk in vitamin D deficient pregnant ladies. This is an economical, safe and easily correctable intervention to combat the harmful conditions like pre-eclampsia and IUGR. Based on the fact that related RCTs are heterogeneous and inconclusive, additional RCTs are stipulated. Screening of vitamin D3 deficiency and its treatment is recommended for favourable obstetric outcomes.

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Statement of authorship

In behalf of all authors, I certify that this is an original manuscript and has not been published as full text and is not under consideration for publication elsewhere. Moreover, to the best of my knowledge and belief, it contains no material previously published or written by another person except where due reference is made.

Conflicts of interest

We have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.02.023.

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