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The effect of vitamin D and calcium supplementation on inflammatory biomarkers, estradiol levels and severity of symptoms in women with postpartum depression: a randomized double-blind clinical trial

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ABSTRACT

Objectives: Postpartum depression (PPD) is a major depressive disorder. Vitamin D deficiency may play a role in PPD pathogenesis. This study was designed to determine the effect of vitamin D and calcium supplementation on the severity of symptoms and some related inflammatory biomarkers in women with PPD.

Materials and Methods: Eighty-one women with a PPD score >12 participated in this study. A total of 27 patients were randomly assigned into three groups (1:1:1 ratio) to receive either 50,000 IU vitamin D3 fortnightly + 500 mg calcium carbonate daily; or 50,000 IU vitamin D3 fortnightly + placebo of calcium carbonate daily, or placebo of vitamin D3 fortnightly + placebo of calcium carbonate daily (placebo group) for 8 weeks. At the baseline and end of the study, the severity score of PPD, levels of 25-hydroxy vitamin D, calcium, tumor necrosis factor-alpha (TNFα), interleukin 6 (IL6) and estradiol were measured.

Results: The PPD score had more reduction in the vitamin D + calcium and vitamin D + calcium placebo groups than that of the placebo group (-1.7 ± 3.44, -4.16 ± 5.90 and 0.25 ± 2.81, respectively; $p = 0.008$). The effect of vitamin D on the PPD score was larger when vitamin D was given alone than given together with calcium ($p = 0.042$ and $p = 0.004$, respectively). No significant differences in estradiol, IL6 and TNFα were observed between the three groups.

Discussion: Vitamin D may be effective in improving the clinical symptoms of PPD; however, the mechanism of the effect might not entirely operate through inflammatory and/or hormonal changes.
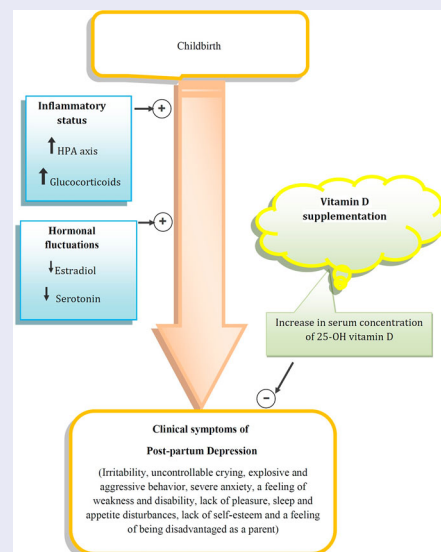
1. Introduction

Postpartum depression (PPD) is a major depressive disorder, which usually begins within four weeks of post-delivery and is usually confined to the 12 months after childbirth. It is estimated that around 20–40% of women become depressed after childbirth [1,2]. PPD symptoms are irritability, uncontrollable crying, explosive and aggressive behavior, severe anxiety, a feeling of weakness and disability, lack of pleasure, sleep and appetite disturbances, lack of self-esteem, and a feeling of being disadvantaged as a parent [3].

Several theories have proposed that PPD is one of the outcomes of hormonal changes and it increases in inflammatory and stress biomarkers post-delivery [4]. During pregnancy, estrogen, and cortisol secretion are increased by the placenta and a sudden eruption occurs after delivery. The result of animal studies shows that estrogen is effective in increasing the neurotransmitter activity that plays a role in increasing the synthesis and reduction of serotonin degradation [5,6]. As a result, the sudden loss of estrogen after childbirth can contribute to the development of PPD by reducing serotonin levels [4]. Moreover, if the hypercortisolism (an occurrence at pregnancy) is sustained post-delivery, it may contribute to an inflammatory response, mood disorder, and PPD [5]. Additionally, history of previous depression, unwanted pregnancy, fear of childbirth, the birth of an abnormal newborn, difficult birth experience, and insufficient economic and social support can increase the risk of PPD [7–9].

In recent years, avoidance of sunlight exposure has led to a decrease in serum 25 hydroxyvitamin D (25[OH]D) levels. Levels of vitamin D are categorized based on serum concentrations of 25[OH]D as sufficient (≥ 75 to < 250 nmol/L), insufficient (≥ 50 to <75 nmol/L), and deficient (<50 nmol/L). Furthermore, vitamin D deficiencies were classified as Mild deficiency (≥ 25 to <50 nmol/L) and severe deficiency (<25 nmol/L) [34]. There is substantial evidence to suggest a minimum desirable level for (25[OH]D) concentrations, 75–100 nmol/L during pregnancy and the early postpartum period [10].

Vitamin D deficiency (VDD) is one of the most prevalent micronutrient deficiencies, and a recent paper reported the prevalence of VDD, 33%, 67%, and 90% among pregnant women in the United States, Iran, and Turkey, respectively [11]. Vitamin D has long been distinguished as a major regulator of calcium homeostasis [11,12]. Moreover, the results of recent studies revealed that vitamin D plays a significant role in brain function and development, and VDD is associated with mood disorders, such as seasonal affective disorder (SAD) and depression [13–17].

There are several biological reasons for the possible role of vitamin D in depression. The presence of 1,25 Di-hydroxyvitamin D in the brain tissue and the presence of vitamin D receptors and alpha-1-hydroxylase (the enzyme which activates this vitamin) in regions of the central nervous system, including hippocampus that is implicated in depression, may explain the role of vitamin D in mood disorder [18,19]. [Boufidou et al. (2009)] have shown that increased concentrations of pro-inflammatory cytokines, including, tumor necrosis factor-α (TNFα) and interleukin- 6 (IL6) were associated with higher rates of PPD. The researchers suggested that immune mechanisms may play a role in the occurrence of PPD [19]. Several biological pathways (e.g. hypothalamic–pituitary–adrenal (HPA) axis, NF-κB and pro-inflammatory cytokines, corticotrophin-releasing hormone (CRH), and serotonin-mediated pathways) have verified the link between vitamin D deficiency and PPD [20].

Pulsed secretion of gonadotropin-releasing hormone (GnRH) releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH), leading to an increase in the level of estradiol. Several laboratory studies have shown that calcium, as a second messenger, induces intracellular signal ions and plays a role in rhythmic stimulation of GnRH [21,22]. After delivery, nutritional demand for calcium is increased [22]. Therefore, vitamin D and calcium supplementation could have beneficial effects on the modulation of sex hormones and inflammatory response that could affect brain function and PPD symptoms.

It has been proposed that PPD is one of the outcomes of change in hormones and inflammatory and stress biomarkers at post-delivery; therefore, vitamin D may play a role in PPD pathogenesis via its effects on inflammatory biomarkers and hormones [4,22].

Based on some cohort studies which have revealed that low serum 25[OH]D concentration is associated with PPD [23–25] and to the best of our knowledge, no available clinical trial in the literature that has evaluated the effectiveness of vitamin D alone and with calcium supplementation on PPD patients; hence, the present study aimed to assess the effectiveness of vitamin D3 alone and with calcium on depression severity, as well as evaluate serum concentrations of 25[OH]D, estradiol, and inflammatory biomarkers, in PPD patients.

2. Materials and methods

2.1. Study participants

Participants included 81 women aged 18–45 years. According to the Diagnostic and Statistical Manual of

Mental Disorders (DSM-IV) [26] and Iranian Edinburgh questionnaire for the diagnosis of PPD whose validity and reliability was confirmed in previous studies, [27] patients were recruited from the outpatient clinic of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, from June to September 2017. They were referred to the psychiatry clinic through the health center and examined by a psychiatrist, and Edinburgh Postnatal Depression Scale (EPDS) was employed to evaluate PPD levels.

Inclusion criteria were scores of more than 12 from the Edinburgh questionnaire, in the postpartum period ranging from 1 to 6 months and body mass index (BMI) less than 35. The exclusion criteria included a value >75 nmol/L (they had sufficient vitamin D and will not benefit from the extra intake of the vitamin), birth abnormalities, taking contraceptive agents, endocrine disorders, previous history of severe depression, and/or other mental disorders and using antidepressants. Moreover, patients with serum calcium concentrations greater than 2.65 mmol/L, intake of vitamin D and calcium supplements during the last six months, having a history of chronic diseases, such as diabetes, renal failure and kidney stones, and gastrointestinal diseases, were also considered as other exclusion criteria.

Due to a lack of similar studies, a study was used for sample size calculation, which was carried out on the effects of vitamin D on the severity of depression in major depressive disorder (MDD) [16]. Based on the changes in the depression severity score, pre- and post-intervention, in the mentioned study (mean 2 = 17.7; mean1 = 13; SD2 = 4.60; SD1 = 4.16), the sample size was calculated as 20 subjects in each group, with a 95% confidence level and 90% power. Then, according to the patients' status and a possible 25% dropout rate during the study, the final sample size was calculated as 27 subjects in each group, (yielding 81 in total).

The protocol of the study was registered in the Iranian Registry of Clinical Trials (www.irct.ir), with this ID: IRCT2016091416123N9.

2.2. Randomization and blinding

The current study was an eight-week randomized double-blind clinical trial with placebo-controlled parallel design. A total of 27 patients were randomly assigned into three groups (1:1:1 ratio), based on a random block allocation method using a computer-generated blocked randomization list and random blocks of six subjects. To ensure that after the random assignment the selection bias did not occur, the allocation was made using allocation concealment by applying two-digit unique codes to each individual. Then, a researcher, who had no clinical participation in the trial, placed the supplements and placebos in numbered bottles based on the random list. Randomization and allocation were hidden from the participants and researchers until the statistical analysis was completed.

2.3. Intervention

Participants were allocated into three groups as follows: 50,000 IU vitamin D3 (D-Vitin 50,000 IU; Zahravi Pharm Co, Tabriz, Iran) fortnightly + 500 mg calcium carbonate (Ca; prepared by School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences) daily; 50,000 IU vitamin D3 fortnightly + placebo of Ca daily and placebo of vitamin D3 fortnightly + placebo of Ca daily for eight weeks. This dosage for vitamin D was designed based on Iran's Ministry of Health and Medical Education recommendation for subjects with vitamin D deficiency [28]. Vitamin D and calcium placebo were similar in shape, size, color, taste, and package to the vitamin D3 and calcium carbonate supplements.

During the intervention, text messages were sent to patients every week to remind them about the intake of supplements and they were contacted every 2 weeks by phone to ask them about any side effects that they might have observed. Patients' compliance was estimated 80% by unused pills that were returned to the researcher.

2.4. The primary and secondary outcomes

The primary outcome was the evaluation of the severity score of PPD, also serum concentrations of 25[OH]D, estradiol and inflammatory biomarkers (interleukin 6 and tumor necrosis factor α) at baseline and at end of the study. The secondary outcomes were anthropometric indices.

2.5. PPD symptoms and severity scoring

For measuring the severity of PPD, the Iranian EPDS questionnaire was used before and after the intervention period. Western studies reported high reliability and validity for the EPDS questionnaire [29,30]. It was translated to Persian and Mazhari and Nakhaee study (2007) shown reliability and validity of 0.83 and 0.76, respectively, for this questionnaire [27].

EPDS questionnaire consisted of 10 items in a multiple-choice format. Each item is scored from 0 to 4, and the sum of these ten items determined the score of PPD severity (range 0–30) [31]. A score above 12 was considered as PPD. The EPDS questionnaire was completed by an interviewer.

2.6. Complementary data

At baseline, a questionnaire, containing demographic data, disease history, using supplements and/or medication use, and history of diet using a three-day food record, was completed. This food record questionnaire determined the amount and type of food eaten in two days of the week and one holiday, before and after the intervention. Nutritionist IV software customized for Iranian foods was used to determine the nutrient intakes, including energy, macro- and micronutrients (vitamin D and calcium) of the participants. Some researchers in Tehran University of Medical Sciences added all Iranian food items to the Nutritionist IV software and these food items were extracted from an Iranian database [32].

Moreover, the physical activity level was evaluated using the short form of the International Physical Activity Questionnaire (IPAQ). A total metabolic equivalent (MET)-minutes/week was equal to walking (3.3METs × min × days) + moderate intensity (4METs × min × days) + vigorous intensity (8METs × min × days), based on the short form of IPAQ [33]. Anthropometric values, such as weight, height, waist, and hip circumference of participants, were measured at baseline and at the end of the study.

2.7. Blood sampling

Five milliliter of fasting blood samples was collected pre- and post-intervention. The blood samples were centrifuged and the sera were frozen and stored at −80°C until the end of sampling.

2.8. Biochemical analysis

Serum 25[OH]D concentration was measured using the enzyme-linked immunosorbent assay (ELISA; Bioactiva diagnostica GmbH, Homburg, Germany; Inter- and intra-assay coefficient variation <7%) and categorized based on the serum concentrations of 25[OH]D as sufficient (≥75 nmol/L), insufficient (≥50 to <75 nmol/L), and deficient (<50 nmol/L) [34]. Total serum calcium concentration was evaluated using a photometric test (Arsenazo III Method; Pars Azmoon Co, Tehran, Iran; intra- and inter-assay coefficient variation <5%; sensitivity 0.15 mmol/L). Serum levels of estradiol were assessed using the enzyme-linked immunosorbent assay (ELISA; Monobind, Calfornia, U.S.A.; intra- and inter-assay coefficient variation <9%) method. ELISA method was also used to measure IL6 and TNFα concentrations (Boster Biological Technology Co., Ltd., U.S.A.). All measurements were done in the central laboratory of the Ahvaz Jundishapur University of Medical Sciences.

According to the kit protocol, assay range for TNFα was between 0.077 and 35.09 nmol/L with intra-assay coefficient variation (CV) <8% and inter-assay CV < 9%, also the assay range for IL6 was between 0.082 and 24.61 nmol/L with intra-and inter-assay CV < 7%.

2.9. Statistical analysis

The normality of data distribution was evaluated using Kolmogorov–Smirnov test. Data with non-normal or normal distribution were shown as median, 25th, and 75th percentile or mean ± standard deviation (SD). One-way ANOVA or parallel non-parametric test (Kruskal–Wallis) in the case of non-normal distribution was used to compare variables between the three intervention groups. Tukey's post hoc test was used to compare the changes in outcome measures between the groups. ANCOVA was used to eliminate the cofactors effects, including age, BMI, and baseline PPD score. The main effects were analyzed again after the adjustment for baseline markers. The analysis was carried out using SPSS version 17.0 (SPSS Inc, Chicago, IL, U.S.A.). For all tests, two-sided p values <0.05 were considered statistically significant. Changes after 8 weeks of intervention (within-group comparison) were compared by paired t-test or Wilcoxon paired rank test (in the case of non-normal distribution) in each group.

3. Results

Of the 81 enrolled subjects, 5 patients failed to complete the study due to personal reasons (one in vitamin D + Ca, one in vitamin D + Ca placebo, and three in the placebo group) and 76 patients completed the study. The flow chart depicts the patients' enrollment in the study (Figure 1).

Based on Table 1, the mean age of the participants was 28.35 ± 1.43 (range 18–45 years). Patients were recruited from June to September 2017. There were no significant differences between the groups in mean weight, height, BMI, age, and physical activity level at baseline. Based on a three-day food record questionnaire, no significant differences were seen in terms of dietary intake of energy, fats, proteins, carbohydrates, vitamin D, and calcium between the three groups (Table 1). Average of 25(OH)D concentration in the whole sample was 37.71 (27.40, 47.69; 25th, and 75th percentile) nmol/L at baseline. The vitamin D status at baseline for each group is shown in Table 2. There were also no significant differences in the baseline concentrations of serum 25[OH]D and calcium between the study groups.

In all groups, the history of the disease and the use of medications were comparable. Results indicate that
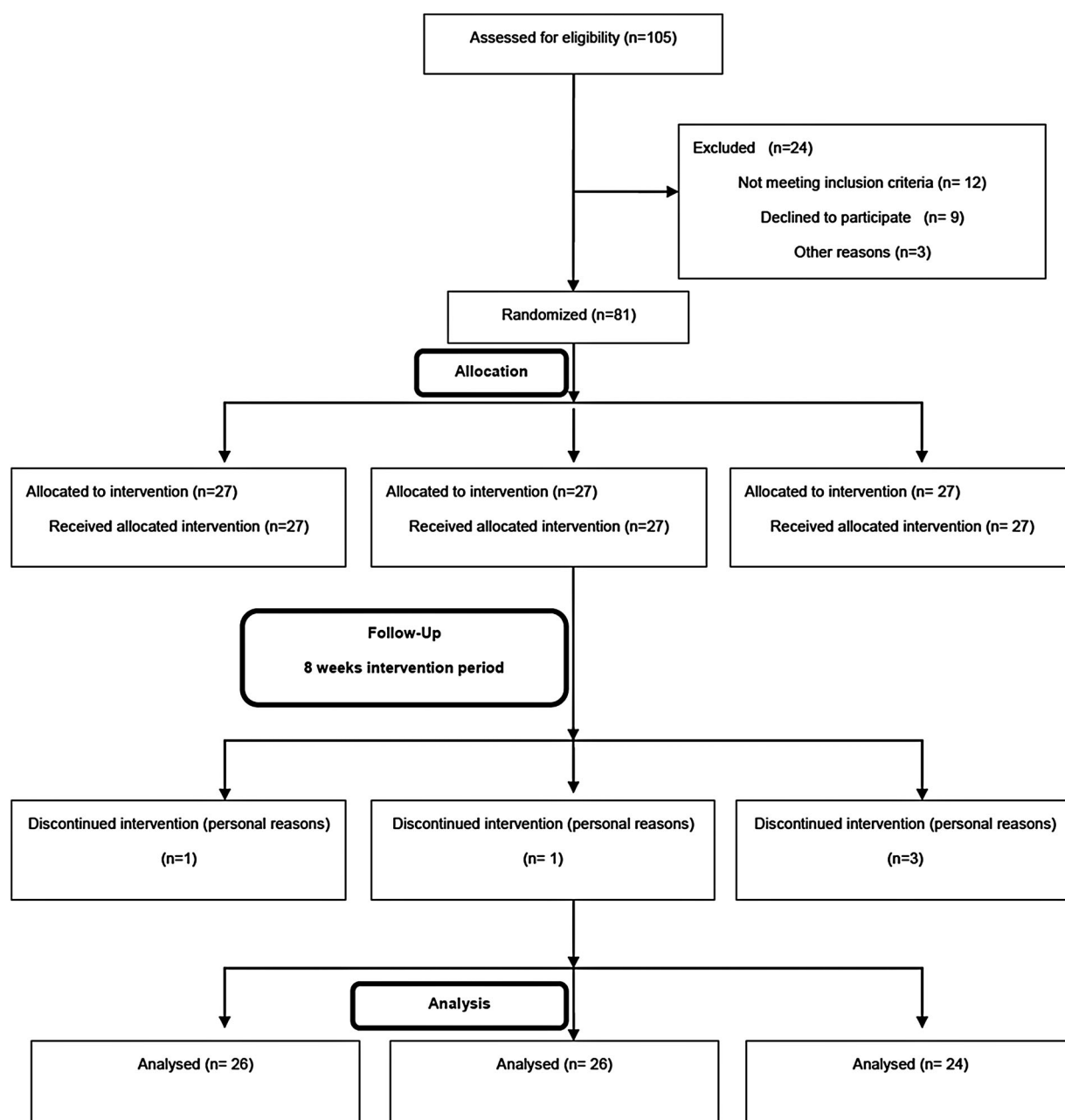
Figure 1. Flowchart of the patients through the study.

vitamin D supplement could reduce the score of PPD; moreover, this reduction was significant between the three groups (Table 2). In vitamin D + Ca placebo group, around 25% reduction in PPD scores was observed. This reduction in PPD score was higher among women who received vitamin D alone, compared to vitamin D + Ca or placebo (Figure 2).

Also, in intervention groups, after 8 weeks of supplementation, serum 25[OH]D concentrations were significantly increased from the baseline. The lowest to the highest range of 25[OH]D concentrations following supplementation was 33.77–70.91 nmol/L in intervention groups. No significant change was observed in the serum concentration of 25[OH]D in the placebo group, as expected. Furthermore, there were significant differences between the serum vitamin D changes in vitamin D + Ca and vitamin D + Ca placebo group when compared to the placebo group, post-intervention (Table 2 and Figure 3). Therefore, the results confirmed that the overall severity of PPD and serum concentrations of 25 [OH]D significantly improved in both vitamin D + Ca and vitamin D + Ca placebo groups; however, greater improvement was observed in the vitamin D + Ca placebo group. The effect of vitamin D on the PPD score was larger when vitamin D was given alone compared to along with calcium.

Table 1. Baseline demographic, anthropometric, and nutritional characteristics in women with PPD.

Characteristics	Vitamin D + Ca (n = 26)		Vitamin D + Ca placebo (n = 26)		Placebo (n = 24)		p-value
Age[a] (years)	26.88	(0.97)	29.25	(1.43)	28.92	(1.62)	0.40
Severity PPD[a] (score)	17.41	(4.10)	17.50	(3.97)	16.43	(3.07)	0.47
Weight[a] (kg)	71.28	(2.32)	68.01	(2.36)	69.19	(2.19)	0.59
Height[a] (cm)	161.55	(0.95)	159.67	(1.19)	158.16	(1.16)	0.10
Waist circumference[a] (cm)	88.41	(2.63)	91.38	(2.33)	88.58	(2.52)	0.64
Waist to hip ratio[a]	0.99	(0.48)	1.01	(0.43)	0.99	(0.05)	0.96
BMI (kg/m²)[a]	27.59	(0.54)	26.62	(0.84)	27.69	(0.86)	0.64
Serum 25(OH)D3[b] (nmol/L)	36.56	(28.67, 46.72)	39.83	(31.94, 47.69)	36.74	(28.40, 45.05)	0.102
Serum Calcium[a] (mmol/L)	2.13	(0.04)	2.13	(0.03)	2.22	(0.04)	0.259
Physical activity[a] (MET-in/week)	156	(0.689)	142.5	(0, 824)	206	(0, 942)	0.471
Energy[a] (KJ/d)	13114.83	(1484.19)	13675.28	(1559.54)	13935.68	(1237.11)	0.17
Dietary protein[a] (g/d)	141.10	(19.77)	144.61	(19.18)	143.10	(18.68)	0.860
Dietary carbohydrate[a] (g/d)	509.18	(56.16)	536.16	(85.55)	553.53	(66.02)	0.134
Dietary fat[a] (g/d)	74.62	(21.78)	77.16	(16.67)	77.30	(17.77)	0.885
Dietary vitamin D[b] (µg/d)	0.93	(0.00, 4.06)	0.28	(0.00, 2.3)	0.69	(0.00, 4.6)	0.243
Dietary calcium[a] (mg/d)	629.44	(342.92)	574.25	(262.26)	595.35	(286.42)	0.273

[a]Data are expressed as mean (standard deviation) and analyzed by one-way ANOVA.
[b]Data are expressed as median (25, 75th percentiles) and measured by Kruskal–Wallis test.
PPD, postpartum depression; BMI, body mass index; MET, metabolic equivalent of the task; KJ, kilojoule.

Between- and within-group changes for inflammatory and hormonal serum levels are shown in Table 2. No significant difference was observed in estradiol, IL6, and TNFα between the three groups.

After supplementation, 25[OH]D concentration in no participants exceeded 125 nmol/L. Compliance rates were 96%, 96%, and 88% in the vitamin D + Ca, vitamin D + Ca placebo, and placebo groups, respectively.

Table 2. Comparisons of the changes from baseline to endpoint measures for the severity of PPD score, 25[OH]D, calcium, estradiol, IL6 and TNFα in vitamin D + Ca, vitamin D, and placebo within and between-group in women with PPD.

| Variables	Vitamin D + Ca (n = 26)	Vitamin D + Ca placebo (n = 26)	Placebo (n = 24)	p-value[d]
PPD[a]				
Baseline	17.41 (4.10)	17.50 (3.97)	16.43 (3.07)	0.478
After intervention period	15.70 (5.15)	13.33 (6.38)	16.68 (5.99)	0.083
Change	−1.70 (3.44) ab	−4.16 (5.90) a	0.25 (2.81) b	**0.008****
p-value[c]	**0.042**	**0.004**	0.586	
Serum 25[OH]D3 (nmol/L)[b]				
Baseline	36.56 (28.67, 46.72)	39.83 (31.94, 47.69)	36.74 (28.40, 45.05)	0.102
After intervention period	51.39 (39.73, 63.04)	58.03 (45.17, 70.91)	42.90 (33.77, 52.04)	0.570
Change	14.43 (5.69, 23.93) a	18.2 (10.63, 25.75) a	6.16 (0.87, 11.43) b	**0.034***
p-value[c]	**0.003**	**<0.001**	0.025	
Serum calcium (mmol/L)[a]				
Baseline	2.13 (0.04)	2.13 (0.03)	2.22 (0.04)	0.259
After intervention period	2.18 (0.17)	2.15 (0.16)	2.12 (0.25)	0.648
Change	0.05 (0.17)	0.02 (0.15)	−0.09 (0.3)	0.332
p-value[c]	0.227	0.529	0.218	
Estradiol (E2) (pmol/L)[b]				
Baseline	156.20 (116.18, 196.17)	269.81 (176.61, 363.06)	202.82 (164.86, 314.16)	0.299
After intervention period	171.94 (131.60, 212.29)	299.66 (196.72, 400.13)	325.65 (138.35, 512.98)	0.163
Change	15.74 (−15.74, 60.64)	29.85 (−127.42, 67.72)	122.83 (−118.31, 302.08)	0.287
p-value[c]	0.468	0.528	0.364	
IL6 (nmol/L)[b]				
Baseline	9.71 (6.34, 13.09)	9.93 (6.31, 13.56)	12.34 (7.58, 17.11)	0.348
After intervention period	10.20 (6.74, 13.66)	10.85 (7.28, 14.41)	12.06 (7.22, 16.91)	0.644
Change	0.49 (−3.24, 6.80)	0.65 (−0.19, 2.14)	−0.28 (−1.89, 1.29)	0.287
p-value[c]	0.493	0.096	0.693	
TNFα (nmol/L)[b]				
Baseline	13.86 (9.49, 18.23)	14.58 (9.40, 19.77)	17.48 (10.19, 24.78)	0.516
After intervention period	15.60 (10.61, 20.60)	14.57 (9.23, 19.90)	17.49 (10.51, 24.47)	0.615
Change	1.74 (−0.80, 4.29)	−0.01 (−1.59, 1.73)	0.01 (−3.73, 3.40)	0.590
p-value[c]	0.166	0.934	0.994	

[a]Data are expressed as mean (standard deviation) and analyzed by one-way ANOVA.
[b]Data are expressed as median (25, 75th percentiles) and measured by Kruskal Wallis test.
[c]Paired sample t-test was used for comparing the baseline, with endpoint values within each group.
[d]ANCOVA was used and adjusted for baseline measures of 25(OH)D and BMI. Tukey posthoc analysis was done and different letters confirm the significant difference.
*p < 0.05, **p < 0.01
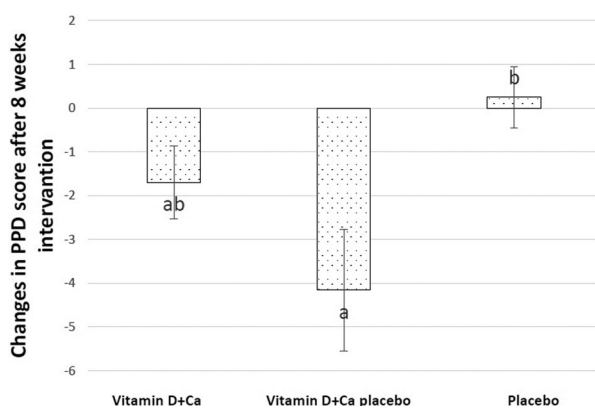
Figure 2. Comparisons of the changes in the severity of postpartum depression (PPD) score after 8 weeks of intervention. Different letters confirm a significant difference. Data are shown as mean ± standard of error.
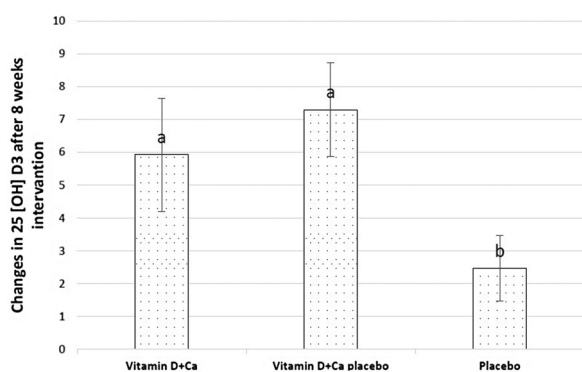
Figure 3. Comparisons of the changes in 25[OH] D3 after 8 weeks of intervention. Different letters confirm a significant difference. Data are shown as mean ± standard of error.

Also, participants did not report any adverse effects such as hypercalcemia. There were no significant differences in terms of serum calcium concentrations between the groups ($p = 0.332$)

4. Discussion

Up to now, six prospective studies have shown that lower 25[OH]D serum concentrations during pregnancy or after delivery are related to the occurrence of PPD in women [23–25,35–37]. To the best of our knowledge, this is the first clinical trial that examined the effect of vitamin D and calcium supplementation on the severity of symptoms in women with PPD. We discovered that vitamin D supplementation with an oral pill containing 50,000 IU vitamin D3 fortnightly for 8 weeks had beneficial effects on the symptom of PPD and serum concentrations of 25[OH]D.

The present study did not find any significant changes for the serum concentrations of estradiol, TNFα, and IL6

in patients with PPD following supplementation. These young women with moderate overweight had high energy consumption but very low calcium and principally vitamin D intake. Regarding the sun exposure, although the blood samples were taken in the summer, 87% of the subjects were not exposed to significant sunlight because of their clothing according to their religion, and also because of using sun protection creams.

The previous study demonstrated that giving 50,000 IU vitamin D fortnightly for 4–6 months had no adverse effects and improved concentrations of serum 25[OH]D [38]. In the present study, participants in the intervention groups received 50,000 IU of vitamin D3 every 14 days; therefore, they received about 3600 IU of vitamin D daily which was less than the UL of vitamin D (4000 IU), and it was an appropriate dosage, according to the Institute of Medicine study [34,39]. Furthermore, this dosage for vitamin D was based on Iran's Ministry of Health and Medical Education recommendation for subjects with vitamin D [28]. Since a daily intake of the supplement may be difficult for these depressed patients, the dose of 50,000 IU/fortnightly, was selected.

In our study, this dose of vitamin D improved vitamin D status, at least from deficient to insufficient in vitamin D + Ca and vitamin D + Ca placebo groups. It had a beneficial effect on the symptoms of PPD, but may not have been sufficient enough to affect the pro-inflammatory cytokines.

In agreement with our results, a study, which assessed the effect of 2000 IU/day vitamin D3 from 26 to 28 weeks of gestation until childbirth in non-depressed women, reaffirmed the possible effects of supplementation in preventing and decreasing the symptoms of perinatal depression [40].

Different results have been reported in studies conducted on MDD patients. In a study on postmenopausal women, supplementation with 400 IU/day vitamin D3 combined with 1,000 mg/day carbonate calcium for 2 years did not improve the symptoms of depression. The different findings with ours may be due to the different pathophysiology of depression in postmenopausal women and the use of different tools to measure depression in the study [15]. In another study, supplementation with 5000 IU/day of cholecalciferol for 6 weeks, despite significant increases in 25[OH]D concentrations, had no significant effects on the symptoms of depression and anxiety [41]. The reasons for this discrepancy in the results could be partly due to the differences in the age of participants, follow-up duration, doses, and type of vitamin D supplementation. In another study, vitamin D supplementation had beneficial effects on mental disorders like MDD and seasonal affective disorder (SAD). In line with our results, in patients with

MDD who received a daily intake of vitamin D + fluoxetine or fluoxetine alone, a greater improvement in depression symptoms was reported in the group receiving vitamin D + fluoxetine after 4 weeks [16]. Antidepressant medication, hormone therapy, and interpersonal therapy may be used for patients with PPD, but patients in our study received none of them during the intervention. Additionally, in a study on patients with MDD, vitamin D supplements showed significant effects on increasing the level of 25[OH]D, insulin and total antioxidant capacity and decreased depression score, compared to the controls [42]. Moreover, in another study that assessed the effect of vitamin D supplementation on SAD showed that supplementation with 400 IU/day of vitamin D had a positive effect on reducing the symptoms of SAD after five days [13].

In a recent systematic review, cohort studies were evaluated in the field of VDD during pregnancy and postpartum, and its relationship with the incidence of PPD was discussed. Prospective studies have shown that low concentrations of 25[OH]D are associated with increased symptoms of PPD in women at post-delivery. No clinical study was reported in this recent systematic review, so we proposed the hypothesis and mechanisms that supplementation with vitamin D may play a significant role in the recovery of PPD [20]. In our study, the findings support the recent systematic review.

A combination of factors is involved in reducing the severity of the PPD. There are some documents about immunomodulation and anti-inflammatory effects of vitamin D [6, 15, 43]. The mentioned systematic review on cohort studies reported that the effect of vitamin D on the pathogenesis of PPD is related to its effect on the adrenal hypothalamus (HPA) axis and the reduction of glucocorticoids through the possible role of calcium in preventing sudden estrogen loss [20].

Borges *et al.* have shown that vitamin D plays a significant role in brain development and is probably involved in the regulation of the brain function [43]. The biological evidence, supporting the hypothesis of VDD's effect on the pathogenesis of PPD, may be through the presence of vitamin D response elements in the promoter of serotonin gene, the presence of vitamin D receptors in the zones of the brain that are disturbed in PPD, [15, 44] and also, the interactions between vitamin D receptors and glucocorticoid receptors in the hippocampus [6].

After delivery, the nutritional demand for calcium is increased in women due to lactation. We expected that calcium along with vitamin D could exert synergic effects in improving the symptoms of PPD, but the results were not observed. Further investigations in future studies could help identify the mechanisms for this finding.

Some researchers have reported higher levels of pro-inflammatory cytokines in PPD subjects [4]. As the results revealed, supplementation with vitamin D3 had an effect on the serum concentrations of estradiol and IL6 in the intervention groups, but this difference was not significant when compared to the placebo group.

In the present study, it remained uncertain that vitamin D can reduce the severity of symptoms of PPD by increasing estradiol and/or decreasing the production of pro-inflammatory cytokines. Moreover, the lack of vitamin D supplementation effect on pro-inflammatory and hormonal factors may be due to the fluctuations of serum levels of these factors in PPD women [4]. Serum or plasma cytokines do not necessarily reflect what is happening at the cellular and this case brain/cell interface, but would suggest that it is not a systemic effect, as measured by those circulating cytokines. Additionally, it could be due to the late improvement of pro-inflammatory and hormonal factors. Possibly, a longer period of supplementation could be effective in reducing levels of pro-inflammatory and hormonal factors. However, since PPD symptoms usually have a short duration, the actual effects of the intervention on these factors could be obscured.

4.1. Strengths and limitations

This clinical trial had some strong points. Firstly, to the best of our knowledge, this study was the first randomized double-blind clinical trial that examined the effect of vitamin D and calcium supplementation on PPD symptoms. Secondly, significant increases in serum 25[OH]D concentrations in the two vitamin D intervention groups indicated that our study had satisfactory compliance and participation. However, this study had some limitations: we only included the mothers who had low levels of serum 25[OH]D (lower than 75 nmol/L) and it is not clear if the same effect would be obtained in mothers with normal serum vitamin D levels. Moreover, the effect of only Ca (without vitamin D) was not studied.

In conclusion, this clinical trial indicated the beneficial effects of one oral 50,000 IU vitamin D3 supplement fortnightly for 8 weeks on the severity and symptoms of PPD in women with vitamin D deficiency. Although the inflammatory and hormonal factors did not change significantly, improvement in the clinical symptoms of PPD was a satisfactory finding for clinical settings. It seems supplementation with vitamin D3

over a longer course may be warranted to assess its effects on pro-inflammatory and hormonal factors.

Author Contributions

All persons who met authorship criteria are listed as authors. S Amini and S Jafarirad contributed significantly to the work's conception, participated in the writing and critical revision of the manuscript in a manner sufficient to establish the ownership of the intellectual content. R Amani contributed significantly to the work's conception. B Cheraghian analyzed and interpreted data. M Sayyah and AA Hemmati involved in the design of work. All authors approved the final version of the manuscript to be published.

Ethics approval

The study protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, in accordance with the Declaration of Helsinki (IR.AJUMS.REC.1396.59, date: March 10, 2017). Written informed consent was given by all the participants before their inclusion in the study. Furthermore, the study design was registered in Iranian Registry of Clinical Trials (www.irct.ir) with this ID: IRCT2016091416123N9.

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