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Research paper

Effects of vitamin D supplementation on depression and some involved neurotransmitters

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ABSTRACT

Background: : Low vitamin D levels are associated with a dysregulated hypothalamic-pituitary-adrenal (HPA) axis and depression but a causal relationship has not been established. This study aimed to evaluate the effects of vitamin D supplementation on depression severity, serum 25(OH)D, and some neurotransmitters in patients with mild to moderate depression.

Methods: : An 8-week double-blind randomized clinical trial was conducted on 56 subjects with mild to moderate depression, aged 43.0 ± 1.15yrs. The patients were randomly allocated into two groups: intervention (50,000 IU cholecalciferol/2wks) and control (placebo). Biochemical parameters (serum 25(OH)D, iPTH, oxytocin and platelet serotonin), and depression severity (Beck Depression Inventory-II (BDI-II[1])) were initially and finally assessed.

Results: : Following intervention, significant changes were observed in the intervention group compared to the controls: 25(OH)D concentrations increased (+40.83 ± 28.57 vs. +5.14 ± 23.44 nmol/L, $P<0.001$) and BDI scores decreased (-11.75 ± 6.40 vs. -3.61 ± 10.40, $P = 0.003$). Oxytocin concentrations were significantly reduced in controls (-6.49 ± 13.69 ng/mL, $P = 0.01$), but between -group differences were insignificant. Within- and between-group differences of platelet serotonin concentrations were not significant; however, the increment in controls was higher (+0.86 ± 10.82 vs. +0.26 ± 9.38 ng/mL, $P = 0.83$).

Limitations: : Study duration may not reflect the long-term effects of vitamin D on depression. It seems necessary to assess tryptophan-hydroxylasetypes1&2 in relation to vitamin D in serotonin pathways.

Conclusions: : Eight-week supplementation with 50,000 IU/2wks vitamin D, elevated 25(OH)D concentration of subjects with mild to moderate depression and significantly improved their depression severity. However, there was no evidence that the anti-depressive effect of vitamin D supplementation is mediated by the measured neurotransmitters.

1. Introduction

Depression is the most prevalent 21th century mood disorder causing undesirable health and economic implications for both the affected individuals and societies (Marcus et al., 2012; Gowda et al., 2015; Gururajan et al., 2016). Antidepressants entail side effects and complications (Li et al., 2014),hence the importance of finding more practical preventive and therapeutic strategies (Wang and Gorenstein 2013a).This requires a better understanding of the mechanisms involved in depression and their interactions (Adibfar et al., 2016; Amini et al., 2018).It is held that a combination of etiological factors contribute to the development of depressive disorders, possibly presenting a wide range of symptoms. Among these symptoms, mention can be made of genetic, immunologic, and environmental factors, biogenic amine deficiency, and neurogenesis. It is likely that HPA axis is the coupling ring between these factors and the development of depression (Jesulola et al., 2018) with serotonin (Cowen and Browning 2015) and oxytocin (Cochran et al., 2013; De Cagna, Fusar-Poli et al. 2019) as major contributors. HPA axis is influenced by seasonality (Pierre et al., 2018) which is accompanied by the predominance of a pro-inflammatory state during cold seasons (Pierre et al., 2016). Along the same line of evidence, the seasonality of mental disorders has been studied with controversial results (Rosen et al., 1990; de Graaf, van Dorsselaer et al. 2005; Winthorst et al., 2011). However, the possible effect of season on mental health has raised the issue of the effect of vitamin D status on mental health and, more specifically, depressive

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[1] BDI-II: Beck Depression Inventory-II

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disorders (Gu et al., 2018; Gu et al., 2019).

It has been shown that active form of vitamin D is produced by many tissues including the brain and also vitamin D receptors (VDRs); also, vitamin D binding proteins (DBPs) have been discovered in the central nervous system (CNS), particularly in areas associated with mood and depression (Kesby et al., 2011; Patrick and Ames 2015; Caldwell et al., 2019). Therefore, the role of vitamin D, as a neuro hormone, is suggested to go beyond the maintenance of calcium homeostasis and bone health (Li et al., 2014) to the growth and development of neuron cells, brain function, synthesis, release and regulation of neurotransmitters, and effect on mood (Gowda et al., 2015; Patrick and Ames 2015; Kesby et al., 2017; Libuda et al., 2017).

It is well known that the low level of serotonin, a tryptophan derived neurotransmitter involved in mood and behavior, is associated with depression (Mottolese et al., 2014; Homan et al., 2015; Raz et al., 2016); furthermore, one of the supposed sub-mechanisms of vitamin D in the pathophysiology of depression is its regulatory role in the gene expression of brain serotonin in a “tissue specific manner” through up-regulating TPH2, a key limiting enzyme of serotonin synthesis pathway (Patrick and Ames 2015; Sabir et al., 2018). However, it is not clear whether vitamin D supplementation affects depression severity through the up-regulation of serotonin in the brain. The results of the limited studies conducted in this context were based on the measurement of serotonin concentration in serum or dry blood samples (Muss et al., 2016; Kim et al., 2018); however, determining the platelet serotonin concentrations, as an exact alternative of serotonin in cerebrospinal fluid (CSF), is more precise and necessary (Audhya et al., 2012; Raz et al., 2016).

It has been reported that oxytocin plays different roles in mood, behavior, sociality, and brain development, and its insufficiency is associated with many mood disorders, including depression (Domes et al., 2016; Sanchez-Vidana et al., 2016; Jobst et al., 2018). There is increasing recognition that production and secretion of nano-peptide oxytocin are dependent on steroid hormones such as vitamin D (Bozdogan et al., 2018; Jirikowski et al., 2018a). On the other hand, recent studies have shown that DBPs exist in the oxytocinergic neurons of brain (Caldwell et al., 2019). This finding might be related to the role of vitamin D as an epigenetic factor in the production, secretion, modulation, and function of oxytocin (Caldwell et al., 2019) through several proposed mechanisms (Hamza et al., 2017). However, to the best of our knowledge, no research has performed RCT to evaluate the effect of vitamin D supplementation on oxytocin concentration levels in patients with depression. To elucidate the magnitude of these mechanisms, more investigations are required.

Several studies have provided evidence for the association of vitamin D and depression, emphasizing that low levels of vitamin D were common in patients with depression (de Koning, van Schoor et al. 2015; Libuda et al., 2017; Aghajafari et al., 2018; Wong et al., 2018; Briggs et al., 2019; Casseb et al., 2019; Ikonen et al., 2019; Jeyaseelan 2019; Mohaddesi et al., 2019; Zhang et al., 2019; Zhou et al., 2019). Nonetheless, RCTs have led to controversial results regarding the effects of vitamin D supplementation on depression severity. Despite some cellular mechanisms proposed for the role of vitamin D in the development of depression (Berridge 2017), the possible link among vitamin D, HPA axis, and depression is yet to be studied. Accordingly, for the first time, we explored the effect of high-dose vitamin D supplementation on depression, neurotransmitters, and HPA axis.

2. Materials and methods

2.1. Study design

The present study was a double-blind RCT conducted on patients with mild to moderate depression and no other psychiatric disease, referred to the outpatient clinics of Baharloo Hospital, Tehran, Iran. The intervention duration was eight weeks, and the study took place between May 2018 and June 2019. The protocol of this trial was previously published in detail (Kaviani et al., 2019).

The inclusion criteria were (1) 18–60 years of age and (2) having mild to moderate depression with no other psychiatric disease according to the psychiatrist's assessments through the structural clinical diagnostic interview based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM–IV) criteria and BDI-II score. In this study, mild to moderate depression was defined as a BDI-II score ranging from13 to 29 (Wang and Gorenstein 2013b).Although incident cases were preferably enrolled, subjects with pre-existing depression were also included in the current research. However, incident and old cases were similarly distributed among the study groups by randomization. It should be mentioned that individuals with severe depression were excluded because they have to be treated with medication, and ethically, supplementing vitamin D alone is not feasible at the stage of practical human research (if he/she is randomly allocated in the intervention group).

The non-inclusion criteria were: (1) Having a history of heart infarction, angina pectoris, stroke, kidney stones, high blood pressure, liver disease, and hyperparathyroidism, (2) pregnancy and/or lactation, (3) reproductive-aged women (under 50 years old) not receiving adequate contraception, (4) consuming nutritional supplements containing vitamin D from two months prior to the intervention. Exclusion criteria were: (1) Lack of willingness to continue the study and (2) failure to follow the interventional program.

Registered volunteers meeting the inclusion criteria and not having the non-inclusion criteria were referred to a psychiatrist for diagnostic tests. The protocol and objectives of the study were explained to eligible participants, and written informed consent was further obtained; afterwards, the subjects were enrolled and randomly allocated into either intervention (50,000 IU cholecalciferol/2wks) or control (placebo) groups according to their entrance code. Vitamin D_3 supplements and placebos were completely similar in appearance and packaging; they were purchased from Zahravi Pharmaceutical Company (Iran) which had no role in any part of the study. Of note, the placebo used in this study was made of human oral paraffin based on the manufacturer's instruction. Moreover, to select a safe dose of vitamin D supplements, safety considerations were based on the upper tolerable intake level of vitamin D for adults (4000 IU day^{-1}) (Patrick and Ames 2015) and the results pertaining to previous studies with higher doses of vitamin D (50,000 IU w^{-1}) (Sepehrmanesh et al., 2016). Therefore, the dose of vitamin D used in this study (50,000 IU/2wks) seemed safe.

On the first study visit (week 0), during a face to face interview, a general socio-demographic questionnaire was completed for each subject; information concerning the history of illness and medication, exposure to sunlight, sunscreen use, tobacco and drug use, alcohol consumption, and physical activity level were further gathered. Next, subjects were asked to stick to their usual diet, physical activity level, and medications during the intervention period, as prescribed by their physician. Anthropometric and blood pressure measurements as well as blood sampling and laboratory investigations were performed on all participants before and after intervention. Depression severity was further assessed through BDI-II questionnaire prior to and following the study.

In the first and second visits (week 4), subjects were given vitamin D supplements or placebos according to their study groups. Neither investigators nor participants were aware of the treatment assignments. To ensure that at least 50% of the pills were consumed, participants were asked to take their pills in the clinic in the first and second study visits. Also, the participants received a reminder call and a written "instruction of use" for the consumption of the remaining pills. They were also asked to return the pills that were not consumed for any reason in their next visit. Therefore, the adherence assessment was done based on supplement/placebo count and patient self-report.

Ethical approval was obtained from the Ethics Committee of

National Nutrition and Food Technology Research Institute (NNFTRI) (IR.SBMU.NNFTRI.REC.1396.185). The clinical trial registration code was obtained from the Iranian Registry of Clinical Trials (IRCTID: IRCT20170926036425N1) and ClinicalTrials.gov (NCT03766074).

2.2. Anthropometric measures and blood pressure

Anthropometric measures including weight, height, waist, and hip circumferences were measured before and after the study; waist to hip ratio (WHR) and body mass index (BMI: weight (kg)/height^2 (m^2)) were further calculated. Systolic and diastolic blood pressures (SBP and DBP) were measured with a digital sphygmomanometer at the beginning and end of the study. More details were previously described (Kaviani et al., 2019).

2.3. Laboratory investigations

Venous blood sample was collected (10 mL; 5 mL in tubes without anticoagulant and 5 mL in tubes with EDTA[2]) from each subject and kept on ice in cold box for transportation to the Laboratory of Nutrition Research, NNFTRI, for laboratory investigations.

Sera separated from clot samples by centrifugation at room temperature (RT) were aliquoted in fresh microtubes and stored at −80 °C until analysis. Platelets were obtained from anticoagulated whole blood samples by two steps of centrifugation; the prepared sample was stored frozen at −80 °C until analysis as described elsewhere (Kaviani et al., 2019). In this study, enzyme immunoassay (EIA) method was employed for assessing all biochemical parameters as follows: serum 25(OH)D (Euroimmun EIA kit, Lubeck, Germany), iPTH (Euroimmun EIA kit, Lubeck, Germany), oxytocin (Zellbio EIA kit, Ulm, Germany), and platelet serotonin (Zellbio EIA kit, Ulm, Germany).

2.4. Outcomes

The primary outcome was the significant elevation of serum 25(OH) D concentration from baseline until the end of intervention. Vitamin D status was categorized based on circulating concentrations of 25(OH)D as: deficiency (<50 nmol/L), insufficiency (50–75 nmol/L), and normal status (>75 nmol/L) (Rosen et al., 2012). Secondary outcomes were significant changes in neurotransmitters, serum oxytocin, and platelet serotonin. Other secondary outcomes were significant changes in serum iPTH and depression severity (BDI-II score) from baseline to the end of the eight-week intervention.

2.5. Statistical analyses

The sample size was calculated based on previous studies (Wang et al., 2013) through considering an effect size of 0.75 and a power of 80%. Afterwards, according to the following formula, a sample size of 56 patients was calculated, with 28 patients assigned to each group (Kaviani et al., 2019).

$$n = \left[\frac{2(z_{1-a/2} + z_{\beta})^2 SD^2}{(\mu_1 - \mu_0)^2} \right]$$

Data were expressed as mean ± SD (standard deviation) to describe the quantitative data and absolute or relative frequencies of the qualitative data. Shapiro–Wilk's test was used to assess the normality of data distribution. Chi-square test was employed to compare qualitative variables between the groups at baseline. Based on the study design, two groups were investigated within two time periods (before and after intervention); thus, paired-sample *t*-test or Wilcoxon test (based on the normality distribution of data) was utilized to compare within-group changes. In order to compare between-group changes, independent sample *t*-test or Mann-Whitney test (based on the normality distribution of data) was used. The significance level was $P<0.05$. Data were analyzed using Statistical Package for Social Sciences (SPSS) software v.21 (SPSS Inc., Chicago, IL, USA).

3. Results

Out of the 69 participants, 56 were incident and 13 were old cases (intervention $n = 6$, control $n = 7$). However, 13 subjects were excluded as they were either not willing to continue the study and/or failed to follow the interventional program. Ultimately, a total of 56 subjects (intervention $n = 28$, control $n = 28$) including 50 women (89.29%) and 6 men (10.71%) aged 43.0 ± 1.15 yrs completed the study (Fig. 1).The estimated adherence of participants was approximately100% because all patients followed the study protocol, completed the study, and referred for post-tests after eight weeks of their entrance time. No statistically significant differences were observed between general characteristics and other parameters of study groups at baseline (Tables 1, , and –3). Furthermore, as observed in Table 3, there were no significant within- and between-group differences in the final values of weight, BMI, waist and hip circumferences, WHR, SBP, and DBP.

Following intervention, vitamin D status was significantly improved only in the intervention group (Table 2); the final 25(OH)D concentration was significantly higher in the intervention group compared with the control group (+40.83 ± 28.57 vs. +5.14 ± 23.44 nmol/L, $P<0.001$)(Table 3). None of the patients complained about adverse drug reactions and there was no report of suicide attempt.

After the intervention, a significant reduction was observed in BDI scores of the intervention group compared to baseline (−11.75 ± 6.40, $P<0.001$) and the control group (−11.75 ± 6.40 vs. −3.61 ± 10.40, $P = 0.003$); however, the BDI score of control group showed no significant changes at the end of intervention (Table 3).

At the end point, the serum oxytocin was significantly reduced in the control group compared to the baseline (−6.49 ± 13.69 ng/mL, $P = 0.01$); however, this reduction was not statistically significant compared to the intervention group (Table 3).

No significant within- or between-group differences were found in platelet serotonin concentrations after the intervention. However, the increase in platelet serotonin concentration in the control group was more than the intervention group following the intervention (+0.86 ± 10.82 vs. +0.26 ± 9.38 ng/mL, $P = 0.83$) (Table 3).

Final iPTH concentrations of both study groups had no statistical difference. However, after intervention, iPTH concentrations significantly increased in the control group compared to the baseline (+5.10 ± 6.002 pg/mL, $P<0.001$) (Table 3).

4. Discussion

This study is the first to perform RCT to evaluate the effects of vitamin D supplementation on platelet serotonin and serum oxytocin, as neurotransmitters purportedly implicated in depression, and also depression severity in depressed patients. Our results showed that eight-week vitamin D supplementation at 50,000 IU/2wks increased serum vitamin D concentration significantly and up-graded the vitamin D status of patients with mild to moderate depression into an acceptable condition. This was accompanied by the significant amelioration of depression severity as compared with the control group. However, this intervention could not significantly influence the serum oxytocin and platelet serotonin concentrations.

There are contradictory results concerning vitamin D sufficiency level but based on serum 25(OH) D concentrations, >75 nmol/L has been reported as vitamin D sufficiency (Patrick and Ames 2015). Although the intake of 600 IU/day vitamin D is recommended for adults by guidelines(Gustafson 2014), current data show that a vitamin D supplementation of 4000 IU d^{-1} is required to reach vitamin D

[2] Ethylene DiamineTetraactic Acid

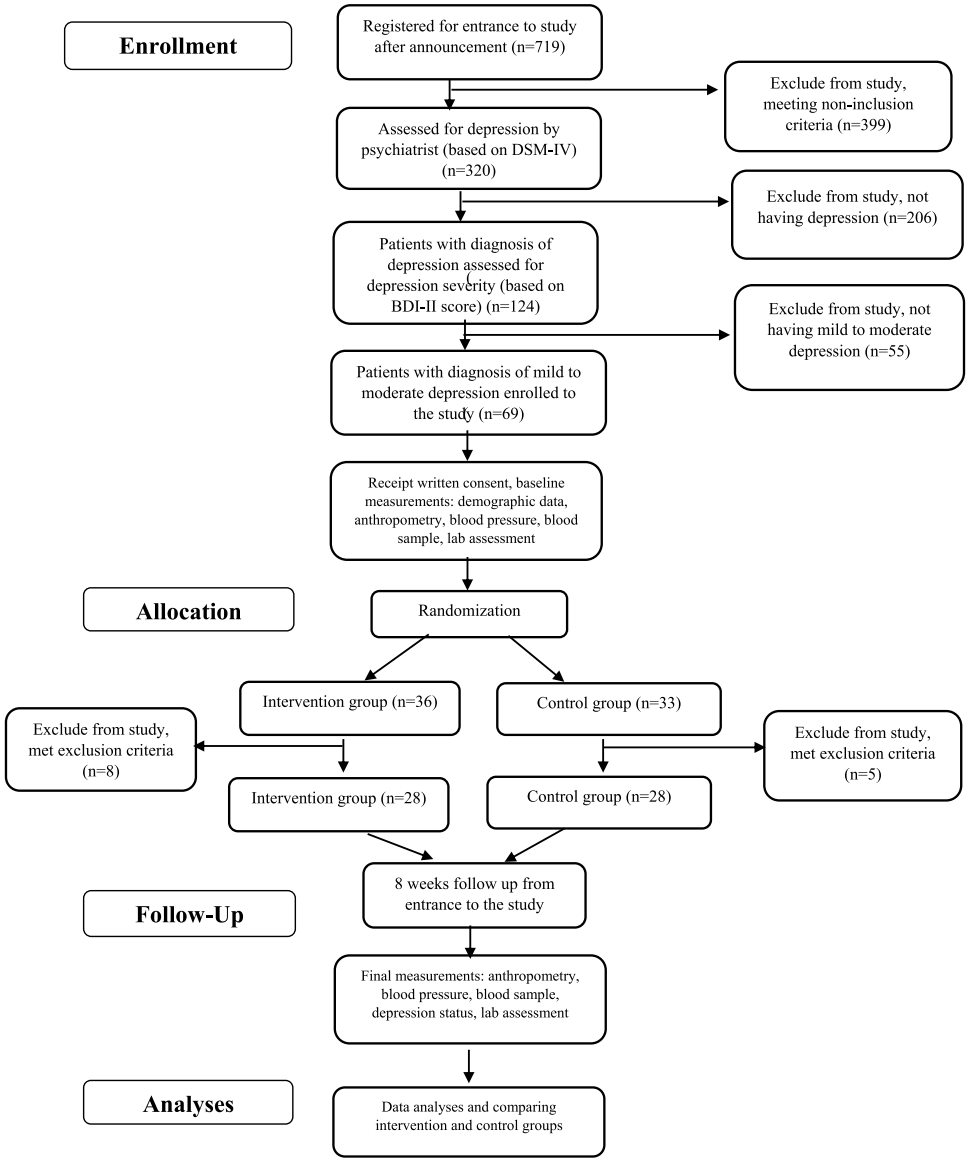

Fig. 1. Summery of the study design.

sufficiency status(Patrick and Ames 2015). In our study, the (~3571 IU d^{-1}) dosage of vitamin D supplement not only could increase serum 25(OH) D concentration and up-regulate vitamin D condition into sufficient, but also entailed no side effects or complaints. These results are in agreement with other studies investigating the effect of vitamin D supplementation on mood disorders (Mozaffari-Khosravi et al., 2013; Sepehrmanesh et al., 2016; Mousa et al., 2018; Jalali-Chimeh et al., 2019).

Recent studies have shown that the active form of vitamin D can be produced in the brain; also, the genes of VDRs are expressed in brain, particularly in areas associated with mood and social behaviors (Kesby et al., 2011; Patrick and Ames 2015; Caldwell et al., 2019). Besides, others have proposed the roles of vitamin D in the regulation of synthesis, release, and function of neurotransmitters and brain function (Bozdogan et al., 2018; Jirikowski et al., 2018b). Our results are consistent with some of the previous studies (Mozaffari-Khosravi et al., 2013; Sepehrmanesh et al., 2016; Ghaderi et al., 2017; Penckofer et al., 2017). However, some studies have failed to indicate any significant improvement in depression status following vitamin D supplementation (Dean et al., 2011; Kjaergaard et al., 2012; Wang et al., 2013; Yalamanchili and Gallagher 2018).It is highly important to examine the vitamin D status of participants prior to the intervention because supplementation with a high dosage of vitamin D (40,000 IU d^{-1}) over six months could not improve the depression status of patients with depression and vitamin D deficiency (Kjaergaard et al., 2012). One possible explanation for vitamin D inefficacy in mood improvement is that such amount of vitamin D could not compensate for hypovitaminosis D and, consequently, the depression severity of patients (Gustafson 2014). Noteworthy, at the beginning of our study, most of the subjects had optimal levels of serum 25(OH)D, possibly hampering the true effects of vitamin D supplementation. However, in spite of the participants having adequate levels at baseline, it was still helpful. This might lead to the conclusion that “more than adequate” vitamin D levels should be targeted in depressed patients. The controversies among different studies may be attributed to the differences in dosage and type of vitamin D supplement, intervention duration, method of supplementation, age of target group, additional simultaneous interventions, and vitamin D supplementation. Additionally, it should be noted that depression is a multi-factorial mood disorder that several factors rather than vitamin D alone are involved in its pathology and more studies are needed to clarify their interactions.

In the present study, supplementation with vitamin D was not able

Table 1
General characteristics of participants at base line.

Variable		Study group (n) Intervention (n = 28)	Control (n = 28)	P* value
Sex †	Female Male	27 (96.4) 1 (3.6)	23 (82.1) 5 (17.9)	0.193
Age (year)‡		43.14 (9.25)	42.86 (8.01)	0.902
Educational level†	Illiterate & elementary Guidance & high school Diploma University	7 (25) 6 (21.4) 7 (25) 8 (28.6)	3 (10.7) 6 (21.4) 7 (25) 12 (42.9)	0.50
marital situation†	Single Married Divorced	2 (7.1) 26 (92.9)	3 (10.7)24 (85.7)1 (3.6)	0.53
Sunlight exposure a day†	No exposure 10–60 min >60 min	8 (28.6)18 (64.3)2 (7.1)	5 (17.9)22 (78.6)1 (3.5)	0.49
Time of sunlight exposure†	10 AM- 15 PM Other times	18 (64.3)10 (35.7)	12 (44.4)15 (55.6)	0.14
Duration of Sunlight exposure†(min)		26.61 (25.09)	24 (16.76)	0.86
Sunlight exposure† (part of body)	Face feet Hand from wrist Hand from arm Combination of above	8 (28.6)1 (3.6)−1 (3.6)18 (64.3)	3 (10.7)−2 (7.1)1 (3.6)22 (78.6)	0.23
Sunscreen usage†	Never Occasionally Often Always	13 (46.4)5 (17.9)2 (7.1)8 (28.6)	13 (46.4)8 (28.6)2 (7.1)5 (17.9)	0.71
Drug usage†	Never Very low Low Moderate High	23 (82.1)3 (10.7)1 (3.6)−1 (3.6)	23 (82.1)3 (10.7)1 (3.6)1 (3.6)-	0.74
Alcohol consumption†	Never Very low Low	26 (92.9)1 (3.6)1 (3.6)	21 (75)6 (21.4)1 (3.6)	0.13
Physical activity level†	Very low Low Moderate High Very high	17 (60.7)9 (32.1)2 (7.1)–	15 (53.6)8 (28.6)3 (10.7)1 (3.6)1 (3.6)	0.67

† Number (%).
‡ Mean (± SD).
* Denotes the significance of differences between the study groups, chi-square test for qualitative data and for quantitative data, independent sample *t*-test (fornormal distribution), Mann–Whitney test (for non-normal distribution).

Table 2
Initial and final vitamin D status of study groups based on circulating concentrations of calcidiol.

Status† Group	Before intervention				After intervention			
	Deficiency	Insufficiency	Sufficiency	P* value	Deficiency	Insufficiency	Sufficiency	P* value
Intervention [n (%)]	1 (3.6)	9 (32.1)	18 (64.3)	0.058	–	1 (3.6)	27 (96.4)	0.005
Control [n (%)]	7 (25)	9 (32.1)	12 (42.9)		4 (14.3)	7 (25)	17 (60.7)	
Total [n (%)]	8 (14.3)	18 (32.1)	30 (53.6)		4 (7.1)	8 (14.3)	44 (78.6)	

† Vitamin D status was defined as: deficiency (<50 nmol/L), insufficiency (50–75 nmol/L) and normal status (>75 nmol/L).
* Denotes the significance of differences in the distribution of vitamin D categories between the study groups (chi-square test).

Table 3
Baseline characteristics and comparison of changes within and between groups after the intervention †.

Group Variable	Intervention (n = 28)			Control (n = 28)			Between-group P** value	
	Before	After	P*value	Before	After	P* value	Before	After
25(OH)D (nmol/L)	87.1 (28.55)	127.92 (24.93)	<0.001	73.64 (31.94)	78.77 (27.05)	0.26	0.10	<0.001
Weight (kg)	75.72 (12.22)	75.94 (12.40)	0.17	75.15 (16.73)	75.12 (17.1)	0.90	0.88	0.51
BMI (kg/m^2)	29.98 (4.64)	30.06 (4.71)	0.15	28.55 (5.33)	28.52 (5.34)	0.84	0.29	0.43
Waist circumference (cm)	98.91(10.54)	99.46 (10.80)	0.12	97.75 (12.27)	97.70(11.89)	0.90	0.71	0.35
Hip circumference (cm)	113.39 (8.29)	113.66 (8.27)	0.10	112.32 (11.70)	112.07 (11.7)	0.43	0.69	0.051
WHR	0.87 (0.07)	0.87 (0.07)	0.76	0.87 (0.07)	0.87 (0.07)	0.39	0.84	0.56
SBP (mm Hg)	122.25 (14.11)	120.04 (11.60)	0.47	120.18 (13.06)	119.64 (13.15)	0.82	0.57	0.65
DBP (mm Hg)	79.79 (10.78)	76.21 (7.58)	0.15	77.75 (10.31)	76.14 (8.88)	0.59	0.52	0.43
Serum iPTH (pg/mL)	13.26 (14.39)	13.43 (9.30)	0.07	11.08 (8.33)	16.18 (7.92)	<0.001	0.64	0.07
Serum oxytocin (ng/mL)	41.26 (15.86)	38.13 (12.42)	0.29	41.09 (20.63)	34.60 (18.55)	0.01	0.90	0.38
Platelet serotonin (ng/mL)	48.71 (8.88)	48.97 (9.45)	0.96	48.65 (12.74)	49.51 (10.50)	0.81	0.98	0.83
BDI-II score	23.86 (5.49)	12.11 (6.12)	<0.001	21.79 (5.74)	18.18 (12.82)	0.053	0.23	0.003

† All values are means (± SDs). 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; iPTH, intact parathormone; BDI-II, Beck Depression Inventory-II.
* Denotes the significance of within-group changes, paired sample *t*-test (for normal distribution), Wilcoxon test (for non-normal distribution).
** Denotes the significance of between-group changes, independent sample *t*-test (for normal distribution), Mann–Whitney test (for non-normal distribution).

to significantly change platelet serotonin concentration. The results of the few previous observational studies on the relationship between vitamin D and platelet serotonin concentrations are equivocal. In agreement with our results, a clinical review reported higher platelet serotonin concentrations in depressed patients with coronary artery diseases (CAD) compared to healthy individuals. The investigators stated that this observation might be due to the differences between serotonin concentrations in patients with depression and healthy subjects (Adibfar et al., 2016). However, the results of another study showed that platelet serotonin concentration in schizophrenic patients with depression was significantly lower than healthy controls and schizophrenic patients without depression, respectively (Peitl et al., 2016). Vitamin D plays two different roles in brain and gut: 1) it activatesTPH2, the key limiting enzyme in serotonin synthesis pathway in brain, resulting in more serotonin production in brain and better mood status; 2)itsimultaneouslyrepressesTPH1in the gut, where 90% of total serotonin production occurs, resulting in less serotonin production in the gut (Patrick and Ames 2015). Therefore, through eliminating the repressive effect of vitamin D on TPH1 in suboptimal situation of vitamin D, more serotonin is generated in the gut and accumulated in platelets, as the main storage location of serotonin in peripheral blood (Patrick and Ames 2015). However, platelet serotonin concentration is

impacted by other such factors as polymorphism in serotonin transporter gene (Levada and Troyan 2018), serotonin transporter activity, extracellular levels of serotonin (Muss et al., 2016),and intestinal micro-flora (Kim et al., 2018). It seems that the existence of other physical or mental disorders, concurrent with depression, influence platelet serotonin concentrations, a matter requiring further investigations.

The results of the current study showed that following eight weeks of vitamin D supplementation, serum oxytocin concentration significantly decreased in the control group compared to the baseline. However, this reduction was not significant compared to the intervention group. Given the existence of DBPs in oxytocinergic neurons of brain (Caldwell et al., 2019), this finding might be associated with the role of vitamin D as an epigenetic factor in production, secretion, modulation, and function of oxytocin (Caldwell et al., 2019)through several proposed mechanisms, including methylation of the whole DNA in brain (Hamza et al., 2017), affecting Ca-dependent oxytocin secretion from neurons (Jirikowski et al., 2018a), gene expression o f oxytocin receptors (Bozdogan et al., 2018) and also fast control on oxytocin function via cellular and molecular mechanisms (Jirikowski et al., 2018b). To the best of our knowledge, this is the first RCT conducted to assess the effects of vitamin D supplementation on serum oxytocin concentrations in patients with depression. Accordingly, we were unable to compare our results with other studies; however, given the significant reduction in the serum oxytocin level in the control group, it is likely that vitamin D supplementation did not increase oxytocin, rather it probably prevented more oxytocin decrement. The results of observational studies about oxytocin level in patients with mood disorders are controversial. Some studies have indicated that patients with mood disorders such asautism, bipolar disorder, and depression have lower levels of circulating oxytocin and there is a negative correlation between plasma oxytocin level and depression (Jobst et al., 2018); on the other hand, others have demonstrated that serum oxytocin level increases in treatment-resistant patients with major depression disorder (MDD) (Sasaki et al., 2016). This discrepancy might be ascribed to the difference in the nature of mood disorders, various characteristics of subjects such as women's menstruation phases and use of different methods for oxytocin measurement (Jobst et al., 2018). Further studies are needed to elucidate the magnitude of this effect.

This study had some limitations. Firstly, the observed effects of the short-term vitamin D supplementation on mood status might not necessarily reflect the long-term effects. In addition, it seems necessary toassessTPH1 and TPH2 in order to clarify more dimensions of serotonin pathways in regard to vitamin D.

There is a great need for further clinical trials with longer durations on depressive patients with vitamin D deficiency both to determine the exact effects of vitamin D supplementation and to follow up subjects to investigate the long-term effects of vitamin Don depression severity. Additionally, to explore the novel pathways of depression pathogenesis, assessment of related pathways such as TPH1 and TPH2 along with serotonin is warranted.

5. Conclusion

In conclusion, the eight-week supplementation with a vitamin D amount of 50,000 IU/2wks significantly increased the serum 25(OH)D concentrations of subjects with mild to moderate depression and remarkably improved their mood status. However, vitamin D supplementation in the current study was not able to significantly alter serum oxytocin and platelet serotonin concentrations in the intervention group. Therefore, we found no evidence that the anti-depressive effect of vitamin D supplementation was mediated by the changes in the measured neurotransmitters.

Our findings can to some extent clarify the ambiguity in mechanisms associated with depression pathophysiology; they also contribute to the future prevention procedures and novel therapeutic targets.

Author disclosure

Author Mina Kaviani: contributed in study design, wrote the protocol and performed the study (sampling, intervention, laboratory tests, data analysis), as well as did the literature searches and wrote the first draft of the manuscript.

Author Bahareh Nikooyeh: contributed in study design and did epidemiological aspects of the study (sample size, statistical tests and managing data analysis).

Authors Hamid Zand and ParichehrehYaghmaei: contributed in study design.

Author Tirang R. Neyestani (corresponding author): contributed in study design, wrote the protocol, as well as managed the laboratory tests and literature searches, modified and finalized the manuscript.

Submission declaration

It is declared that the work described has not been published previously and it is not under consideration for publication elsewhere. Also publication of manuscript is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Limitations

- Study duration may not reflect long-term effects of vitamin D on depression.
- Baseline optimal levels of serum 25(OH)D of subjects may hamper the true effects of vitamin D.
- Assessment of tryptophan-hydroxylase (types1&2) in relation to vitamin D in serotonin pathways seems necessary.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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