Journal of Affective Disorders 314 (2022) 143–149

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

A randomized, double-blind, placebo-controlled, 12-week trial of vitamin D augmentation in major depressive disorder associated with vitamin D deficiency

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ARTICLE INFO

Keywords:
Vitamin D deficiency
Vitamin D supplementation
Major depressive disorder
Escitalopram
Antidepressant
Randomized controlled trial

ABSTRACT

Background: Randomized controlled trials (RCTs) of vitamin D (VitD) supplementation for depression have yielded inconsistent results. We conducted the first RCT of VitD supplementation with multipoint serum 25(OH)D assessments in major depressive disorder (MDD) patients with concurrent severe VitD deficiency.
Methods: We randomized antidepressant-free depressed adults with mean baseline 25(OH)D of 11.5 ng/ml to VitD (60,000 IU every 5 days; n = 31) or placebo (n = 28) for 12 weeks. All patients also received escitalopram (10–20 mg/day). Patients were rated at baseline and at the end of weeks 4, 8, and 12. Serum 25(OH)D was estimated at baseline, week 8, and week 12.
Results: In an intent-to-treat analysis, mean Hamilton Depression Scale scores dropped from 25.7 to 5.7 and from 25.8 to 5.0 in VitD and placebo groups, respectively (primary outcome; P = 0.92). VitD and placebo groups did not differ on other objective and subjective ratings of depression, or on global ratings. Similar findings characterized completer analyses. No significant correlations were observed between 25(OH)D levels and depression ratings across the course of the study. Importantly, endpoint escitalopram doses were 4 mg/day higher in placebo than in VitD patients, and 4 mg/day higher in VitD deficient than in VitD sufficient patients.
Limitations: A ceiling effect with escitalopram may have prevented the discovery of benefits with VitD supplementation.
Conclusions: VitD supplementation does not improve antidepressant outcomes with flexibly dosed escitalopram. VitD deficient depressed patients may require higher antidepressant doses to experience benefits similar to those whose deficiency is corrected by VitD supplementation.

1. Introduction

Several studies have identified the presence of vitamin D, vitamin D receptors and enzymes (CYP 24A1, CYP 27B1) involved in the formation of calcitriol in key brain regions implicated in the pathophysiology of depression (Eyles et al., 2005; Kalueff and Tuohimaa, 2007). Vitamin D is suggested to play a neuroprotective role in the brain through its effects on inflammation and the downregulation of the proinflammatory cytokines that are associated with depression (Buell and Dawson-Hughes, 2008; Song and Wang, 2011; Zittermann et al., 2004). Vitamin D is also suggested to modulate the association between the inflammatory response and depression through its effect on the immune system (Menon et al., 2020; Mora et al., 2008).

Given this background, several investigators have examined the therapeutic effects of vitamin D supplementation in major depression. The pooled results were inconclusive (Gowda et al., 2015; Vellekkatt and Menon, 2019); this may be due to methodological limitations of the trials, such as a failure to enrich samples with subjects with vitamin D deficiency (Menon et al., 2020). Additionally, the relationship between change in serum vitamin D levels and depression scores is unclear because post-trial vitamin D status was not assessed in many positive (Vellekkatt et al., 2020) as well as negative (Choukri et al., 2018; Jorde et al., 2008) trials.

The prevalence of vitamin D deficiency is high in India across age and baseline health status strata (Aparna et al., 2018; Beloyartseva et al., 2012). To our knowledge, only two prior Indian studies have assessed

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https://doi.org/10.1016/j.jad.2022.07.014
Received 24 August 2021; Received in revised form 4 July 2022; Accepted 9 July 2022
Available online 16 July 2022

the effects of vitamin D supplementation in patients with major depression (Nebhinani et al., 2017; Vellekkatt et al., 2020); only one of them was a randomized controlled trial (RCT) with change in depression scores as the outcome of interest (Vellekkatt et al., 2020); both showed beneficial effects of supplemental treatment.

Given the limited data in this field, the high prevalence of depression as well as of vitamin D deficiency in the general population in India, and the likely wide acceptability of vitamin D supplementation as a therapeutic approach, we conducted this 12-week RCT with the following methodological strengths: we studied only subjects with concurrent vitamin D deficiency, and we assessed the vitamin D status of subjects during and after the study. Our primary objective was to assess the effect of add-on oral supplementation with vitamin D on the 12-week change in depression symptom ratings in patients with major depressive disorder and vitamin D deficiency. One secondary objective, among others, was to correlate the serum vitamin D levels with depressive symptom ratings across the course of the study.

2. Materials and methods

2.1. Study design and setting

The study was a randomized, double-blind, placebo-controlled trial conducted from August 1, 2020 to December 31, 2020, in the psychiatry outpatient clinic of IQRAA International Hospital and Research Center, a multi-specialty tertiary care hospital in Kozhikode, Kerala, India. The protocol of the study was approved by the Institutional Ethics Committee. The trial was registered in the Clinical Trials Registry of India (CTRI/2020/08/027428). Written informed consent was obtained from all subjects.

2.2. Sample

Subjects were outpatients, aged 18 to 65 years, diagnosed with major depressive disorder without psychotic features, based on the Diagnostic and Statistical Manual of Mental Disorders-5 criteria (American Psychiatric Association, 2013), and with a score $\geq$ 15 on the 17-item Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960). Patients were recruited only if they had no antidepressant or antipsychotic exposure in the past 2 months and if their serum 25-hydroxy vitamin D (25[OH]D) level was <20 ng/ml, indicating deficiency (Holick et al., 2011). Patients were excluded if they had suicidal ideation, major comorbid psychiatric disorders such as posttraumatic stress disorder, comorbid substance use disorder, or comorbid unstable medical disorder. Pregnant and lactating women were also excluded.

2.3. Study procedures

Serum 25(OH)D levels were obtained in all potentially eligible consenting patients (n = 140), and those whose levels were <20 ng/ml (n = 59) were centrally randomized by a pharmacist, using a computer-generated random number sequence, to receive, once in 5 days, identical-appearing capsules of either vitamin D (60,000 IU; n = 31) or placebo (starch; n = 28). All patients additionally received escitalopram 10 mg/day, uptitrated (depending on efficacy and tolerability) to a maximum of 20 mg/day, by a psychiatrist who was blind to the treatment allocation. Oral clonazepam was permitted in a maximum dose of 2 mg/day for patients who reported anxiety or insomnia. No other psychotropic medications were used during the trial. Treatments were continued until the end of the 12-week study, after which subjects received treatment as usual.

2.4. Assessments

Depression severity was assessed using the 17-item HAM-D (primary outcome measure), the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979), and the Beck Depression Inventory (BDI) (Beck et al., 1961) (secondary outcome measures). Patients were also assessed using Clinical Global Impression-Severity (CGI-S) and Clinical Global Impression-Improvement (CGI- I) (Guy, 2000). Ratings were obtained at baseline and at the end of weeks 4, 8, and 12 by raters who were blind to the treatment allocation.

Blood samples were collected after 8–10 h of fasting at baseline, week 8, and week 12, and 25(OH)D levels were measured by the ARCHITECT 25-OH Vitamin D assay, a quantitative, automated chemiluminescent microparticle immunoassay (CMIA). It utilizes a four-parameter logistic curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve. The measuring interval of the ARCHITECT 25-OH Vitamin D assay is 3.4 to 155.9 ng/ml. The highest observed limit of quantitation (LoQ) value at $\leq$20 % coefficient of variability was 2.4 ng/ml (6.0 nmol/l). We preferred the ELISA method of assay over high performance liquid chromatography with mass spectroscopy method due to cost constraints. Treatment adherence was assessed through pill counts for escitalopram and placebo capsules, while it was presumed from changes in serum 25(OH)D levels for vitamin D.

2.5. Statistical methods

We planned to recruit 60 patients with approximately 30 patients in each group. Our RCT therefore had 80 % power to detect a moderate to large effect size of approximately 0.75 with alpha for statistical significance set at $P < 0.05$ (Norman et al., 2012).

The intent to treat (ITT) sample was defined as all patients who were randomized and who received at least one dose of study medication. Missing values were imputed using the last observation carried forward method. A completer analysis was also planned.

The primary outcome was the ITT comparison of the improvement between baseline and 12-week endpoint in HAM-D scores in vitamin D vs control groups. The comparison of response and remission rates between vitamin D and control groups was a secondary outcome; in this context, response was defined as at least 50 % attenuation of HAM-D scores between baseline and endpoint, and remission as an endpoint HAM-D score of 7 or less.

Other secondary outcomes were ITT comparisons of 12-week improvements between baseline and endpoint in MADRS, BDI, CGI-S, and CGI-I ratings. A special secondary outcome was the assessment of whether change in 25(OH)D levels was correlated with change in depression ratings. Completer analyses were also secondary outcomes We also examined the proportion of patients whose 25(OH)D levels transitioned to sufficiency, defined as a level of at least 30 ng/ml (Kennel et al., 2010). Finally, we compared endpoint escitalopram doses between vitamin D and placebo groups to ascertain whether differences in clinical improvement between groups, if any, may have been due to inequities in escitalopram dosing.

Normality of data was assessed by the Shapiro-Wilk test. The independent sample *t*-test (or the Mann-Whitney test, where distributions were non-normal) and the chi-square test were used to compare continuous and categorical variables, respectively. Spearman's rho was used to examine correlations between continuous variables where distributions were non-normal. Changes in clinical variables between study baseline and endpoint were compared between groups using 2 × 4 two-way repeated measures multivariate analysis of variance (RMANOVA); the group x time interaction was the statistic of interest. Alpha for statistical significance was set at 0.05.

3. Results

3.1. Sample description and disposition

The description and disposition of the sample are presented in Table 1 and Fig. 1, respectively. There were 140 patients who met the

Table 1
Demographic and clinical description of the sample at baseline.

Variable	Vitamin D (n = 31)	Placebo (n = 28)	Comparison
Age (years)	34.9 (10.5)	39.3 (11.8)	t = 1.51, P = 0.14
Baseline vitamin D level (ng/ml)	11.6 (2.6)	11.4 (3.3)	t = 0.28, P = 0.78
Male	10 (32.3 %)	6 (21.4 %)	χ^2 = 0.87, P = 0.35
Education (years)	11.9 (3.1)	10.9 (3.4)	t = 1.24, P = 0.22
Employed	24 (77.4 %)	25 (89.3 %)	χ^2 = 1.47, P = 0.22
Marital status (single)	26 (83.9 %)	23 (82.1 %)	χ^2 = 0.03, P = 0.86
Positive family history of psychiatric illness	18 (58.1 %)	11 (39.3 %)	χ^2 = 2.08, P = 0.15
Baseline HAM-D score	25.7 (8.0)	25.8 (10.3)	t = 0.05, P = 0.96
Baseline MADRS score	36.4 (11.5)	33.1 (10.2)	t = 1.14, P = 0.26
Baseline BDI score	25.3 (10.3)	27.9 (12.8)	t = 0.85, P = 0.40

Values presented are mean (standard deviation) or frequency (percentage).
Abbreviations: BDI=Beck Depression Inventory; HAM-D=Hamilton Depression Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale.

study selection criteria and who consented for participation in the study. Screening for 25(OH)D levels identified 61 patients with vitamin D deficiency (<20 ng/ml). Of these, two withdrew consent, leaving 59 patients for randomization.

The mean (standard deviation) (M [SD]) age of the 81 excluded patients was 43.4 (11.1) years. The excluded sample was 38.3 % female (n = 31). The M (SD) 25(OH)D levels in these patients was in the insufficient range, at 23.4 (3.2) ng/ml. The excluded patients were older (t = 4.41, df = 138, P < 0.001) and less likely to be female (χ^2 = 16.41, df = 1, P < 0.001). Expectedly, they had significantly higher 25(OH)D levels (t = 22.64, df = 138, P < 0.001).

The M (SD) age of the randomized sample was 36.8 (11.3) years. The sample was 72.1 % female. The M (SD) 25(OH)D level was 11.5 (2.9) ng/ml. The average patient was severely depressed at baseline (mean HAM-D score, nearly 26). These patients were randomized to receive vitamin D (n = 31) or placebo (n = 28) augmentation of escitalopram treatment. There were no baseline differences between vitamin D and placebo groups in sociodemographic or clinical variables (Table 1).

Forty-one patients completed the 12-week study; of the 18 dropouts, 7 and 8 patients completed their 8-week and 4-week assessments, respectively (all outcome measures were available for them till these time points); 3 patients dropped out of the study before the 4-week assessment time point. Seven patients dropped out of treatment in the vitamin D group and 11 patients dropped out in the placebo group (Fig. 1). The proportion of dropouts did not differ significantly between treatment and control groups (χ^2 = 1.94, df = 1, P = 0.16). In the vitamin D group, 4 patients withdrew consent because of lack of benefit, 2 withdrew consent without stating reasons, and 1 patient did not return for follow up for unknown reasons. In the placebo group, 2 patients withdrew consent because of lack of benefit, 1 withdrew consent for unknown reasons, 4 dropped out because of the experience of adverse effects, and 4 did not return for follow up for unknown reasons. There were no serious adverse events.

No significant differences were observed between the completer and lost to follow up groups on most demographic and clinical parameters at baseline (Table 2; more data available from authors on request). However, the intervention group had a higher proportion of patients with a positive family history of psychiatric illness.

3.2. Treatment

Adherence to escitalopram treatment was confirmed by pill counts; all patients for whom repeated ratings were available showed >90 % adherence. Adherence to the vitamin D treatment protocol is described in the next section.

Cumulated across the course of the study, patients received a median clonazepam dose of 9.8 vs 10.5 mg/day in placebo vs vit D groups, respectively (P = 0.83). At the end of the study, the M (SD) dose of escitalopram in the ITT sample was 13.2 (4.8) vs 15.4 (5.1) in vit D vs placebo groups, respectively (P = 0.10). These values were 12.9 (4.6) vs 17.1 (4.7) mg/day in the completer sample (t = 2.80; df = 39; P = 0.008), a difference of about 4 mg/day.

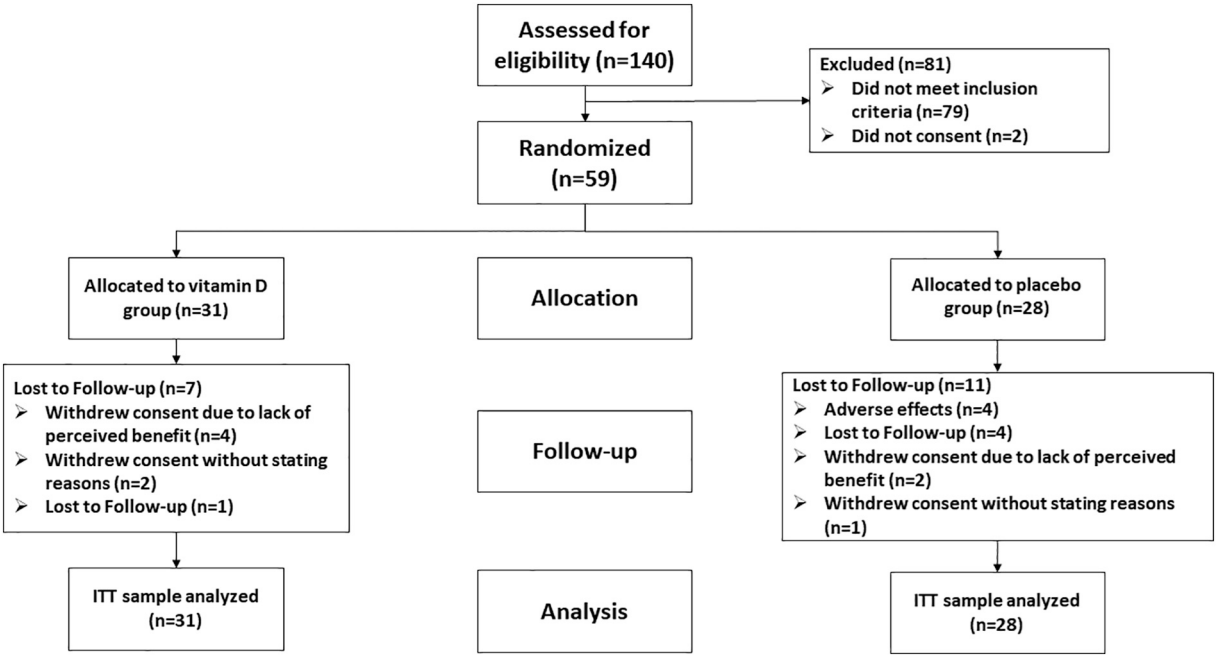

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram for the trial.

Table 2
Comparison of completers and dropouts.

Variable	Completers (n = 41)	Dropouts (n = 18)	Comparison
Age (years)	37.3 (11.4)	36.4 (11.3)	t = 0.85, P = 0.40
Baseline vitamin D levels (ng/ml)	11.4 (3.0)	11.7 (2.8)	t = 0.37, P = 0.71
Male	9 (22.0 %)	7 (38.9 %)	χ^2 = 1.82, P = 0.18
Education (years)	11.6 (3.0)	10.9 (3.8)	t = 0.72, P = 0.48
Employed	34 (82.9 %)	15 (83.3 %)	χ^2 = 0.00, P = 0.97
Marital status (single)	33 (80.5 %)	16 (88.9 %)	χ^2 = 0.63, P = 0.43
Positive family history of psychiatric illness	24 (58.5 %)	5 (27.8 %)	χ^2 = 4.74, P = 0.03
Last administered dose of escitalopram	14.6 (5.0)	13.3 (4.8)	t = 0.92, P = 0.36
HAM-D baseline score	26.2 (9.2)	24.8 (8.8)	t = 0.53, P = 0.60
MADRS baseline score	34.1 (11.1)	36.3 (10.8)	t = 0.70, P = 0.49
BDI baseline score	25.2 (12.0)	29.4 (10.1)	t = 1.28, P = 0.21

Values presented are mean (standard deviation) or frequency (percentage).
Abbreviations: BDI=Beck Depression Inventory; HAM-D=Hamilton Depression Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale.

3.3. 25(OH)D levels

The M (SD) 25(OH)D levels at baseline, week 8, and the 12-week study endpoint are presented in Table 3. Whereas the levels were similar in the two groups at baseline, they were, expectedly, significantly higher in the vitamin D than in the placebo group at both week 8 and week 12. A total of 21 intervention group patients (67.7 %) had a change in 25(OH)D status (from deficient to insufficient or sufficient) across the course of the trial as compared with 5 (17.9 %) control group patients; this difference was statistically significant ($\chi2 = 14.85$, df = 1, P < 0.001). Among study completers, 18 had vit D level <30 nmol/l, 1 had <50 nmol/l, 4 had 50–75 nmol/l, and 18 had levels >75 nmol/l.

Table 3
Vitamin D levels, depression, and clinical severity of illness scores across the study in intervention versus control groups.

Ratings	Vitamin D (N = 31)	Placebo (N = 28)	t or U, df, and P
Vitamin D baseline	11.6 (2.6)	11.4 (3.3)	0.28, 57, P = 0.78
Vitamin D week 8*	73.0 (42.6)	28.9 (37.1)	138.00, 46, P = 0.002
Vitamin D week 12†	77.9 (40.6)	33.6 (41.2)	103.00, 39, P = 0.008
HAM-D baseline	25.7 (8.0)	25.8 (10.3)	458.50, 57, P = 0.71
HAM-D week 4	15.7 (8.9)	14.6 (10.5)	494.50, 51, P = 0.36
HAM-D week 8	7.8 (7.3)	6.2 (4.9)	469.00, 46, P = 0.60
HAM-D week 12	5.7 (6.9)	5.0 (5.5)	470.50, 39, P = 0.93
MADRS baseline	38.0 (11.4)	32.9 (10.1)	1.15, 57, P = 0.25
MADRS week 4	21.5 (12.2)	18.4 (13.0)	509.50, 51, P = 0.25
MADRS week 8	8.9 (8.5)	7.1 (4.8)	470.50, 46, P = 0.58
MADRS week 12	5.5 (16.0)	4.0 (11.5)	441.50, 39, P = 0.91
CGI-S baseline	5.0 (0.7)	4.8 (0.8)	0.85, 59, P = 0.40
CGI-S week 4	3.5 (0.9)	3.5 (1.1)	414.00, 51, P = 0.68
CGI-S week 8	2.3 (0.9)	2.2 (1.0)	348.50, 46, P = 0.43
CGI-S week 12	1.8 (0.8)	1.9 (0.9)	198.50, 39, P = 0.87
CGI-I week 4	3.1 (1.1)	3.0 (1.2)	405.50, 51, P = 0.79
CGI-I week 8	2.0 (1.2)	1.8 (0.8)	309.00, 46, P = 0.61
CGI-I week 12	1.8 (1.2)	1.7 (0.9)	199.50, 39, P = 0.89

Values presented are mean (standard deviation); HAM-D=Hamilton Depression Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale; CGI-S=Clinical Global Illness-Severity scale; CGI-I=Clinical Global Illness-Improvement scale; data available for *48 and †41 patients.

We expected that, with the vitamin D treatment protocol that we followed, 25(OH)D levels would increase by at least 10 ng/ml in the vitamin D group and by less than this value in the placebo group. To our surprise, at treatment endpoint, we found that 7 of 28 placebo group completers exceeded this threshold and 10 of 31 vitamin D group completers did not reach this threshold. This suggests that some of the placebo group patients may have taken vitamin D supplements out of protocol, and that some of the vitamin D group patients may not have taken their prescribed capsules. Because the patterns of rise in vitamin D levels in the placebo and vitamin D groups were contrary to our expectations based on the treatment protocol, one may expect that it would have influenced the change in primary outcome measure (HAM-D). In this context, our cross-sectional examination of the correlation between 25(OH)D levels and depression ratings assumes importance.

At baseline, 25(OH)D levels did not correlate significantly with baseline depression ratings using HAM-D (rho = −0.150, P = 0.26), MADRS (rho = −0.147, P = 0.27), or BDI (rho = −0.124, P = 0.35). Similarly, no significant correlations were observed at 8 weeks with HAM-D (rho = −0.157, P = 0.29), MADRS (rho = −0.108, P = 0.46), or BDI (rho = 0.069, P = 0.64). Finally, no significant correlations were observed at the 12-week study endpoint with HAM-D (rho = 0.098, P = 0.54), MADRS (rho = 0.106, P = 0.51), or BDI (rho = 0.122, P = 0.45).

In a post hoc analysis, driven by our findings of higher escitalopram dosing in the placebo group, we observed that, among study completers, endpoint M (SD) doses of escitalopram were significantly higher (t = 2.48, df = 37, P = 0.018), by about 4 mg/day, in patients with deficient vitamin D levels (n = 16, M [SD] = 16.9[4.8] ng/ml) as compared to those with sufficient vitamin D levels (n = 23, M [SD] = 13.0[4.7] ng/ml).

In another post hoc analysis, we observed that, among study completers, change in HAM-D scores across the study was not significantly different (t = 0.614, df = 39, P = 0.543) between patients whose vitamin D levels did (n = 26, M [SD] = −21.5[9.3]) versus did not rise (n = 15, M [SD] = −19.5[10.3]) by at least 10 ng/ml.

3.4. Improvement in HAM-D scores in vitamin D vs placebo groups

HAM-D changes across the course of the study are presented in Table 3 and Fig. 2. Vitamin D was not associated with significant benefit in either ITT (primary outcome; Pillai's trace = 0.009, F = 0.17, df = 3,55, P = 0.92) or completer (Pillai's trace = 0.021, F = 0.27, df = 3,37, P = 0.85) analyses.

In the ITT sample, the response and remission rates were 77.4 % (n = 24/31) vs 75.0 % (n = 21/28) ($\chi2 = 0.05$, df = 1, P = 0.83) and 58.1 % (n = 18/31) vs 64.3 % (n = 18/28) ($\chi2 = 0.24$, df = 1, P = 0.62) in the vitamin D vs placebo groups, respectively.

In the completer sample, the response and remission rates were 91.7 % (n = 22/24) vs 88.2 % (n = 15/17) (Fisher's exact test, P = 1.00) and 75.0 % (n = 18/24) vs 82.4 % (n = 14/3) (Fisher's exact test, P = 0.71), in the vitamin D vs placebo groups, respectively.

3.5. Improvement in MADRS and BDI scores in vitamin D vs placebo groups

Changes in MADRS and BDI across the course of the study are presented in Table 3. Vitamin D was not associated with significant improvement in MADRS scores in either ITT (Pillai's trace = 0.020, F = 0.37, df = 3,55, P = 0.77) or completer (Pillai's trace = 0.034, F = 0.429, df = 3,37, P = 0.734) analyses. Likewise, vitamin D was not associated with significant improvement in BDI scores in either ITT (Pillai's trace = 0.013, F = 0.24, df = 3,55, P = 0.87) or completer (Pillai's trace = 0.007, F = 0.09, df = 3,37, P = 0.967) analyses.

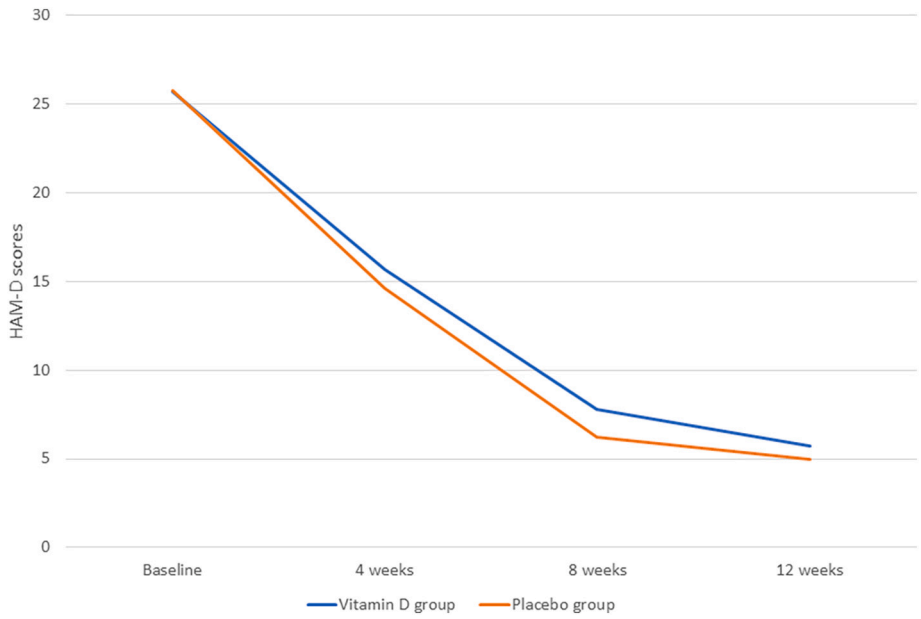

Fig. 2. Hamilton Depression Rating Scale (HAM-D) scores across study period.

3.6. Changes in CGI-S and CGI-I scores in vitamin D vs placebo groups

Changes in CGI-S and CGI-I across the course of the study are presented in Table 3. Vitamin D was not associated with significant improvement in CGI-S scores in either ITT (Pillai's trace = 0.044, F = 0.85, df = 3,55, P = 0.48) or completer (Pillai's trace = 0.100, F = 1.37, df = 3,37, P = 0.27) analyses. Likewise, vitamin D was not associated with significant improvement in CGI-I scores in either ITT (Pillai's trace = 0.028, F = 0.80, df = 2,56, P = 0.46) or completer (Pillai's trace = 0.021, F = 0.41, df = 2,38, P = 0.67) analyses.

3.7. Effect of escitalopram dosing on improvement in depression ratings

The last dose of escitalopram was significantly higher (by 4.2 mg/day) in placebo as compared with vitamin D patients in the completer sample. We therefore conducted a post hoc analysis to understand the implications of escitalopram dosing on the primary endpoint: improvement in HAM-D ratings. In a multivariable linear regression analysis with change in HAM-D scores as the dependent variable and group, baseline HAM-D score, and last dose of escitalopram (mg/day) as the independent variables, only baseline HAM-D score predicted clinical improvement in the ITT sample as well as in the completer sample ($P < 0.001$ in both analyses). This result did not change when endpoint 25 (OH)D levels were used in place of the grouping variable. The implication, therefore, is that the extent to which patients recovered was not driven by vitamin D augmentation or by variations in escitalopram dose.

Further, to examine a possible interaction effect between endpoint antidepressant dose and group on the primary outcome, we repeated the regression analysis, this time, adding a centered interaction term (group x escitalopram endpoint dose) as an additional independent variable. The effect of this interaction variable on change in HAM-D scores was not significant (B = 0.22, 95 % CI for B -0.72 to 1.15, P = 0.645).

4. Discussion

4.1. Summary of main findings

We examined whether vitamin D augmentation of flexibly dosed escitalopram improves antidepressant outcomes in patients with moderate to severe MDD associated with laboratory-confirmed severe vitamin D deficiency. We found that, on all clinical outcome measures, and at all assessment points in this 12-week study, vitamin D augmentation did not outperform placebo augmentation with regard to either speed or magnitude of improvement. We additionally found that 25(OH)D levels did not correlate with subjective and objective depression ratings at any point during the course of the study. However, we did find that patients whose vitamin D deficiency was left uncorrected (because they received placebo) received significantly higher doses of escitalopram, by a mean of about 4 mg/day. These findings suggest that the correction of vitamin D deficiency is not essential for clinical recovery in antidepressant-treated MDD and that, as a novel finding in the research literature, patients with vitamin D deficiency can recover provided that they receive adequate (read higher) doses of antidepressant medication; in our study, escitalopram.

4.2. Strengths of our study

Our study had many important strengths. One strength is that whereas we planned to recruit patients with vitamin D deficiency (25[OG]D levels <20 ng/ml), our sample was actually enriched for patients with severe deficiency; the mean 25[OH]D level was 11.5 ng/ml at baseline. This means that if correction of vitamin D deficiency is important for antidepressant efficacy, it should more easily be ascertained in a sample that is severely deficient.

We examined 25(OH)D levels not only at baseline and endpoint, but also during the course of the study. This allowed us to track whether increases in 25(OH)D levels correlated with clinical improvement. This also permitted us to examine in post hoc analyses clinical outcomes in patients in whom 25(OH)D levels rose substantially vs failed to rise. The post hoc analyses were important because it appeared that some patients in the vitamin D augmentation group may not have been adherent to the augmentation regimen, and some patients in the placebo group may have taken vitamin D out of protocol. To our knowledge, no other study has done this.

Finally, we dosed escitalopram flexibly, based on efficacy and tolerability, in a study of adequate duration (12 weeks); this is what allowed us to discover that even patients who remain vitamin D deficient may respond to antidepressant treatment provided that they receive higher antidepressant doses. We acknowledge, however, that this was an unexpected observation in a secondary analysis that needs to be confirmed in future studies.

4.3. Limitations of our study

We powered our study to identify an effect size of 0.75 based on ratings on a continuous measure as opposed to a proportion, such as response rates to an antidepressant. This means that our study was not powered to detect a smaller advantage for vitamin D, if any. However, given the negligible difference in mean scores between groups at treatment endpoint (Table 3), it is unlikely that a clinically meaningful difference between augmentation and placebo groups exists. It is possible that the vitamin D group patients were underdosed with escitalopram and that they may have improved more had they received escitalopram doses that were comparable with those in the placebo group. Whereas this possibility cannot be ruled out, it must be kept in mind that the response and remission rates in the 2 groups were already substantial, and it is unlikely that these rates could have been much further improved. In this context, one wonders whether a ceiling effect prevented the discovery of differences between groups, if a true difference exists. A considerable proportion among intervention group did not show rise in vitamin D levels as expected; on the other hand, a considerable proportion among placebo group showed unexpected increase in vitamin D levels. We did not perform a sensitivity analysis excluding these patients as the sample size was small. This may also be justified because the cross-sectional analyses showed very low and nonsignificant correlations between 25(OH)D levels and HAM-D ratings at all time points. The study was not monitored as per Good Clinical Practice (GCP) guidelines as, in India, GCP monitoring is not required for investigator-initiated, industry-independent trials. Finally, our findings can only be interpreted in the context of escitalopram-treated depression; because antidepressants vary in efficacy, and because escitalopram is among the more effective antidepressants (Andrade, 2018), it is possible that vitamin D augmentation in deficient patients may have greater clinical impact in patients prescribed less effective antidepressants.

In this trial, vitamin D supplementation was started at the same time as flexibly dosed escitalopram, as opposed to studying the effects of supplementation in non-responders. Here, we were guided by prior studies in this area that showed a beneficial effect of add-on vitamin D supplementation in patients with acute, clinically significant depression (Mozaffari-Khosravi et al., 2013; Vellekkatt et al., 2020). Besides, there have been suggestions to improve practice by employing augmentation or combination approaches during initial treatment in MDD to enhance remission, and possibly, treatment retention rates (Fava and Rush, 2006). Such an approach may prevent clinical progression, and, possibly, neuroprogression associated with non-response.

4.4. Examining the findings in the context of the existing literature

Prior reviews on the role of vitamin D in depression show an association between vitamin D deficiency and depressive symptoms (Anglin et al., 2013; Cuomo et al., 2017); however, in intervention studies, results have been inconsistent (Li et al., 2014; Shaffer et al., 2014). Possible reasons for variation in results include differences in sample (clinical depression vs subsyndromal depression), study setting and design, age range of participants, dose of vitamin D administered, mode of administration, duration of study, and the instruments used to measure outcomes.

Many of the intervention studies had limitations in their design; trials were not restricted to vitamin D deficient subjects, participants were not clinically depressed, or the randomization procedure was unclear (Menon et al., 2020; Spedding, 2014). Three RCTs found moderate to large benefits with vitamin D supplementation (Khoraminya et al., 2013; Mozaffari-Khosravi et al., 2013; Sepehrmanesh et al., 2016). However, all of these RCTs emerged from a single geographical location. There was also sample and treatment heterogeneity in all of these; two of the trials were not restricted to vitamin D deficient subjects (Khoraminya et al., 2013; Sepehrmanesh et al., 2016) while the third trial (Mozaffari-Khosravi et al., 2013), though carried out exclusively on vitamin D deficient subjects, did not involve concurrent use of antidepressant treatment. The study by Sepehrmanesh et al. (2016) has recently been retracted due to concerns about the validity of the data (https://pubmed.ncbi.nlm.nih.gov/33974698/).

Two recent RCTs reported no beneficial effect of vitamin D on the outcome of depression (Choukri et al., 2018; Wang et al., 2016); however, neither of these enriched the sample for clinically depressed subjects with vitamin D deficiency. Vitamin D supplementation has been suggested to help only in patients with concurrent vitamin D deficiency and major depression (Choukri et al., 2018; Menon et al., 2020; Vellekkatt and Menon, 2019); however, although our patients met these criteria, we found no evidence of additional antidepressant benefits with supplementation. Nevertheless, one may still add vitamin D in these patients, if only to correct comorbid vitamin D deficiency.

5. Conclusions

Vitamin D supplementation substantially increases 25(OH)D levels in vitamin D (severely) deficient patients with MDD but does not materially improve antidepressant outcomes with flexibly dosed escitalopram; however, vitamin D deficient depressed patients may require higher antidepressant drug doses to experience benefits similar to those whose deficiency is corrected by vitamin D supplementation. Correction of vitamin D deficiency is desirable for general health but may not be necessary for antidepressant efficacy.

Funding

This work was unfunded.

CRediT authorship contribution statement

PNSK and CA designed the study. PNSK conducted the study. VM and CA were responsible for data analysis. PNSK, VM, and CA drafted the manuscript. All authors contributed to the final version of the manuscript.

Conflict of interest

None. The authors have no conflicts to declare with regard to the contents of this manuscript.

Acknowledgements

We thank Mr. Jaison Joy, Ms. Thoohima Menon, and Ms. Binsha Thandampunathil for assistance with patient recruitment and data collection.

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