Original Article

Effect of Vitamin D Supplement on Mood Status and Inflammation in Vitamin D Deficient Type 2 Diabetic Women with Anxiety: A Randomized Clinical Trial

Abstract

Background: Vitamin D plays an important role in nervous health and depression. Vitamin D deficiency and anxiety affect diabetic status. The purpose of this study was to determine the effect of vitamin D supplementation on anxiety, depression, and inflammation in diabetic women with anxiety. **Methods:** In this randomized controlled trial, totally 51 women with type 2 diabetes (T2DM) and vitamin D deficiency were randomly allocated to receive one oral pearl of 50,000 IU vitamin D3 (26 women) or a placebo (25 women) fortnightly for 16 weeks. Anthropometric indices, sun exposure, dietary intake, depression, anxiety, and stress scores and biochemical biomarkers including high sensitivity C-reactive protein (hs-CRP) and interleukin-10 (IL-10) were measured at the baseline and after 16-week supplementation. **Results:** Mean ± SD age of participant was 47.43 ± 9.57 years old. Baseline values were not different between the groups. Anxiety score changes were significantly lower in vitamin D group than the controls (P = 0.001). Within group comparison indicated that depression in supplement group with lower vitamin D levels was significantly reduced. Serum hs-CRP reduced (P = 0.01), while IL-10 concentrations increased (P = 0.04) in the intervention group. **Conclusions:** Vitamin D supplementation can improve mood status and anti-inflammatory biomarkers in female diabetics with anxiety and vitamin D deficiency.

Keywords: *Anxiety, diabetes, inflammation, Vitamin D, women*

Siavash Fazelian, Reza Amani[1], Zamzam Paknahad, Soleiman Kheiri[2], Leila Khajehali[3]

Department of Clinical Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran, [1]Department of Clinical Nutrition, School of Nutrition and Food Science, Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Epidemiology and Biostatistics, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran, [3]Internal Center, Imam Ali Hospital of Farokhshahr, Social Security Organization, Shahrekord, Iran

Address for correspondence:
Prof. Reza Amani, Department of Clinical Nutrition, School of Nutrition and Food Science, Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: r_amani@nutr.mui.ac.ir

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Introduction

Recently, in addition to the function of vitamin D on calcium metabolism, bone, proliferation, differentiation, and immune modulation, few evidence indicates that vitamin D plays an important role in the brain, nervous system health, and depression.[1] Both vitamin D deficiency and insufficiency show global burden[2] that is more prevalent in women.[3]

The anxiety disorders include some of the most common and disabling psychiatric illnesses. Anxiety is characterized by excessive and persistent worry that can reduce the ability to perform normal activities in life.[4]

A systematic review and meta-analysis has indicated that diabetes is related to increased risk of anxiety disorders.[5] Uncontrolled blood sugar is associated with anxiety and the rates of generalized anxiety disorder (GAD) is higher in diabetes.[6]

Tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) are increased in anxiety due to inflammation and psychoneuroimmunologic pathways.[7] Anxiety can weaken the immune system[8] while some studies show that diabetic patients have higher levels of inflammatory markers such as CRP, interleukin-1 (IL-1), IL-6, TNF-α, interstitial cellular adhesion molecule-1, and vascular cellular adhesion molecule-1.[9] On the other hand, evidence suggests that both depression and anxiety stimulate inflammation in diabetic patient.[10]

Previous interventions have shown the impact of vitamin D deficiency on depression,[11] however, limited studies have been carried out to illustrate the association of anxiety disorders with serum levels of vitamin D.[12] Penckofer *et al.* reported 6-month intervention with 50,000 IU vitamin D significantly decreased depression and anxiety in type 2 diabetes mellitus (T2DM) who had depression.[13]

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Supplementation with vitamin D reduced anxiety levels in premenstrual syndrome in adolescents who had severe vitamin D deficiency.[14] At present, there have been no studies regarding the effect of vitamin D supplementation in women with T2DM affected by anxiety. Therefore, the main aim of this study was to examine the potential impact of vitamin D on anxiety in diabetic women with vitamin D deficiency.

Methods

Participants

In the present randomized double-blind placebo-controlled clinical trial, 64 women with T2DM were recruited from Shahr-e-Kord Diabetes Clinic, Iran. The participants were T2DM women between 20 to 60 years old diagnosed with mild, moderate, or severe anxiety. All participants had vitamin D deficiency or insufficiency [10 (ng/mL) ≤ serum vitamin D ≤30 (ng/mL)]. Diagnosis of T2DM was on the bases of the World Health Organization guidelines.[15]

Anxiety was determined by the Depression, Anxiety and Stress Scales (DASS-21) questionnaire apply for clinical and non-clinical populations that had previously been validated in Iranian population.[16] Each question of DASS-21 has four scales (0 = did not apply to me at all, 1 = applied to me to some degree or some of the time, 2 = applied to me to a considerable degree or a good part of the time, and 3 = applied to me very much or most of the time) to determine the symptoms of anxiety and depression. The sum of scores for each subscale should be multiplied to 2 to evaluate original depression anxiety stress scale of 42 items. Patient's scores on DASS-21 scale can run between 0 to 42. Cut-off points for anxiety score are 0–7, 8–9, 10–14, 15–19, and more than 20 to classify patients into normal, mild, moderate, severe, and extremely severe state of anxiety, respectively.[17,18]

General characteristics of the participants including age, educational levels, suffering from other diseases, duration of T2DM, and medication history were collected through face-to-face interview pre-intervention.

Patients that had neurological or psychiatric disorders, took any medications for depression or vitamin D/multivitamin supplements during the last 4 month, consumed alcohol, were pregnant, or lactating were excluded. The purpose of the study was explained for eligible participants. Women who had the aforementioned criteria and agreed to participate in the study completed informed consent form before commencing the intervention.

Study design, randomization, and blindness

Current study was a randomized double-blind placebo-controlled clinical trial. Block randomization (with block sizes of two) was performed using the Random Allocation Software: (RAS). Participants were randomly allocated into either intervention or placebo group [Figure 1]. The intervention and placebo groups received 50,000 IU cholecalciferol and paraffin soft gel capsules, respectively, fortnightly for 16 weeks. Since normal upper limit of vitamin D intake is 10,000 IU/day, the recommended dosage was regarded safe.[19]

All participants and investigators including the laboratory staff were blind to randomization. Placebo was similar to vitamin D soft gels in color and size and they were presented in dark bottles (A, B) coded by a subject who was not involved in any procedures of the study. Both supplements were produced by Zahravi Pharm Co; Tabriz, Iran.

Sun exposure time

At the beginning and end of the study, sun exposure rate of participants were assessed through a validated questionnaire. Duration of exposure to sunlight [the average minutes (min)/hours (h) of a usual day in the previous week] was asked using a questionnaire.[20] The duration of sun exposure was classified as 0–10 min; 10 min–1 h; 1–2 h; and >2 h. Furthermore, the time of exposure to sunlight during a day was also asked and classified as follows: 7 am–10 am; 10 am–3 pm; and 3 pm–5 pm. At the end of the questionnaire, the part of the body that was exposed to sunlight was asked from all participants. Because of the intense sunlight, the highest score was the exposure of sunlight at 10–3 o'clock.[21]

Dietary assessment

Dietary intakes were assessed by a single researcher using a valid 3-day food record (2 working days and 1 weekend) at pre- and post-intervention. Nutritionist IV software program (First Databank Inc., Hearst Corp., San Bruno, CA, USA) modified for Iranian food was used for dietary analysis.

Physical activity assessment

The physical activity level of participants was determined using the short version of International Physical Activity Questionnaire (IPAQ) validated for Iranians.[22] at pre- and post-intervention.

Anthropometric indices assessment

Anthropometric indices such as height (by a graded wall to the nearest of 0.1 cm), weight (fasting weight was measured with the accuracy of 0.1 kg using Seca scales), waist circumferences (WC) was measured at the midway between lower rib and iliac crest, and hip circumferences (HC) was recorded using standard methods[23] at the baseline and post-intervention by a trained nutritionist. Body mass index (BMI) was calculated by dividing weight (kg) to square height (m^2).

Sample size determination

Considering 0.05 as type 1 error and 0.2 as type 2 error (power = 80%) based on previous study and using

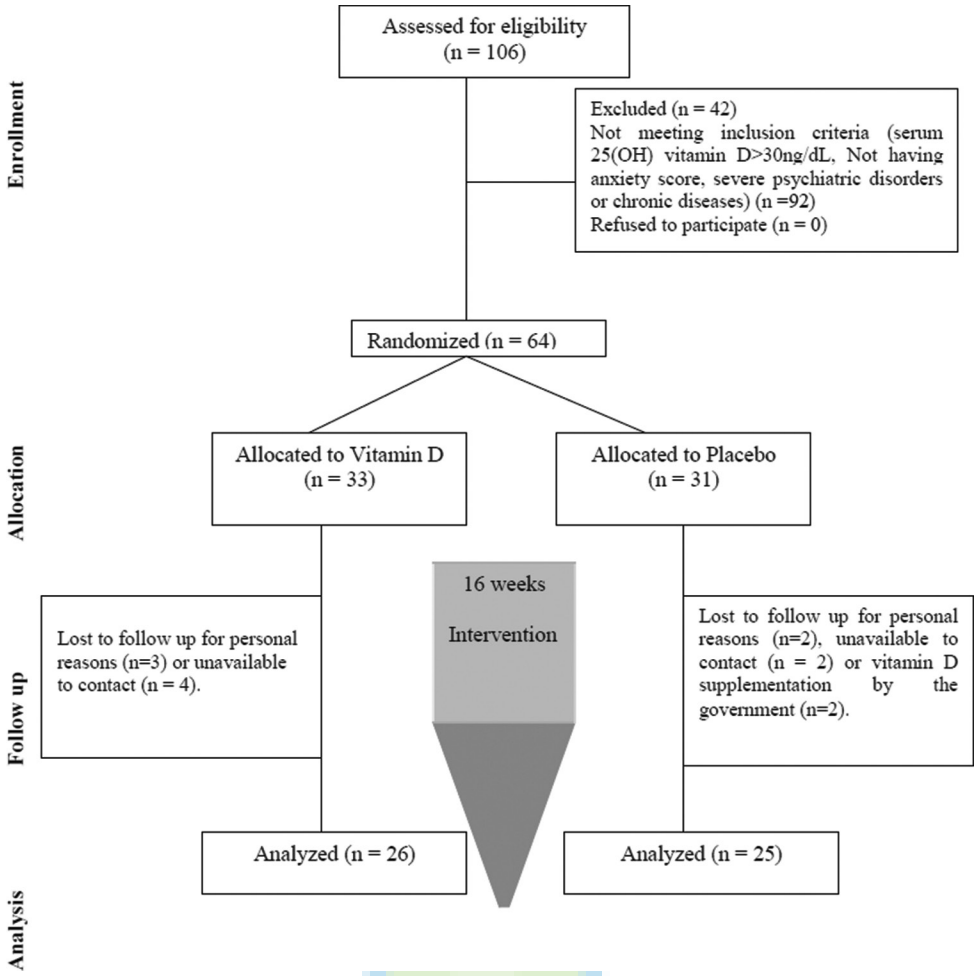

Figure 1: Algorithm of study participants in the two groups

standard deviation equal to 1 and change in mean equal to 0.81 of high sensitivity CRP (hs-CRP), 24 subjects were needed in each group. We added 10% to sample size because of covering possible dropouts to reach 25 participants in each group.[24]

Laboratory analyses

A total of 10 ml of fasting blood samples were collected from each participant after 12 h fasting at the beginning and at the end of study. The blood samples were centrifuged to separate serums and they were then stored at −80°C until further analysis. Serum 25-hydroxy vitamin D levels were quantitatively measured using the enzyme-linked immunosorbent assay (ELISA) method (Monobind ELISA kit, USA). Serum hs-CRP concentrations were assayed using ELISA kit (Diagnostics Biochem Canada Inc, Ontario, Canada).

Statistical analysis

We analyzed data using the SPSS software, version 17 (SPSS Inc, Chicago, IL). The Shapiro–Wilk test was performed to evaluate the normality of the distributions. The between-group comparisons for baseline characteristics were done using independent *t*-test or Mann–Whitney U test for variables with normal and non-normal distribution, respectively. Wilcoxon test and paired *t* tests were applied for within-group analysis. We used analysis of covariance (ANCOVA) test adjusted for baseline values and confounders such as fat and vitamin C intake to compare the two groups pre- and post-intervention. *P*-values <0.05 were considered statistically significant. Between- and within-group analyses of subgroups were performed with the similar methods used previously.

Ethical consideration

The present study was carried out in accordance with the guidelines described in the Declaration of Helsinki.[25] It was approved by the Medical Ethics Committee of Isfahan University of Medical Sciences (Reg. No: IR. MUI. REC.1395.3736). This study has been registered in the Iranian registry of clinical trials (http://www.irct.ir) under the number IRCT20170927036451N1.

Results

Sixty-four TDM2 women with mean ± SD age of 47.43 ± 9.57 years were included to our study. Finally, 51

participants (26 patients in the vitamin D and 25 patients in the placebo groups) completed the trial [Figure 1]. Demographic information including education, BMI, physical activity, sun exposure, waist-to-hip ratio, and serum 25-hydroxy vitamin D at the beginning and post-intervention showed no significant differences [Table 1]. Compliance rate of taking soft pearls was >90% in the two groups.

Analysis of 3-day food records showed no significant differences in terms of nutrients and energy intake, except for fat (P = 0.02) and vitamin C (P = 0.02) at the baseline [Table 2].

Anthropometric variables, sun exposure, and physical activity of both the groups did not change after intervention.

The mean ± SD of anxiety score at the beginning of the study in supplement group was 12.31 ± 4.26 which indicated moderate anxiety. At the end of intervention in vitamin D group, mean ± SD of anxiety severity was 9.15 ± 4.54 this average is classified as mild anxiety level.

Significant reduction in anxiety score was seen in vitamin D group vs. control group (P = 0.001), [Table 1].

Depression changes were not different significantly in vitamin D group vs. controls (P > 0.05), while the depression score significantly decreased post-intervention in vitamin D group, that determined by within-group analyses [Table 3]; (P = 0.03).

Patients were classified into two subgroups as 10> serum vitamin D ≥20 and 20> serum vitamin D ≥30. Subgroup analysis indicated that anxiety score decreased in supplement group in both categories of serum vitamin D (P < 0.5). Depression score in supplement group with lower vitamin D levels was significantly reduced (P = 0.04). Stress did not change significantly in any of the groups and also in vitamin D subgroups [Table 4]. Reduction in hs-CRP levels was significant in patients who took vitamin D supplement in both categories (P = 0.01).

Discussion

Our study showed that 16 weeks supplementation with 50,000 IU vitamin D decreased anxiety, hs-CRP and increased IL-10 concentrations in T2DM women with anxiety for a period of 4 months. Recently Penckofer *et al*. reported that vitamin D supplementation for 6 month can decrease depression and anxiety in diabetic patient with symptoms of depression.[13] Several studies have shown that vitamin D deficiency is associated to poorer mental health.[26,27] However, overall effect of vitamin D in a meta-analysis reported no significant reduction in depression after supplementation. It is worthy to note that most studies examined the individuals with low depression and sufficient serum levels of vitamin D at the baseline.[28]

Vitamin D supplementation reduced anxiety in this trial. Bičíková *et al*. showed that lower serum level of calcidiol was related to anxiety in patients with anxiety disorders assessed by Clinical Global Impressions Scale (CGI).[29] Ataie-Jafari have indicated that anxiety, poor quality sleep, depression, and worry were related to vitamin D deficiency in Iranian adolescences.[30] A trial by Dean *et al*. indicated that vitamin D has no therapeutic effects on anxiety and depression in healthy adult.[31] A study carried out on the effect of daily supplementation with dosage of 5000 IU vitamin D in young adults did not show any changes in anxiety scores,[31] however, another study reported that anxiety improved in diabetic patients received 50,000 IU vitamin D on weekly basis for 6 month.[13]

Table 1: Baseline characteristic of participants in the study groups

Variables	Supplement group (n=26)	Placebo group (n=25)	P
Age (year)	48.5±7.58[a]	46.32±11.16[a]	0.42[†]
FBS (mg/dL)	166.63±63.63	158.32±54.14	0.38[†]
Education, n (%)			
Primary/high school	18 (69.40)	13 (52.00)	0.42*
Diploma	4 (15.30)	7 (28.00)	
University degree	4 (15.3)	5 (20.00)	
Exposure to sunlight score	31.02±24.67[a]	32.54±6.71[a]	0.85[†]
BMI (kg/m^2)	30.21±4.42[a]	29.19±6.41[a]	0.16[†]
Waist-to-hip ratio	0.94±0.05	0.91±0.10	
Physical activity (MET/hours*day)	31.48±18.12[a]	30.21±33.05[a]	0.09[†]
Use of sunscreen cream, n (%)			
Never	14 (53.84)	14 (56.00)	0.92[†]
Sometimes	7 (26.92)	5 (20.00)	
Very often	3 (11.54)	3 (12.00)	
Always	2 (7.70)	3 (12.00)	
25-hydroxy vitamin D (ng/mL)	21.32±5.85[a]	20.73±6.47[a]	0.37[†]

[†]By independent t-test, *By Chi-square test, [a]Mean±SD. SD=Standard deviation, FBS=Fasting blood sugar, BMI=Body mass index, MET=Metabolic equivalent of task

Table 2: Comparison of dietary intake, anthropometric indices, and physical activity in the study groups pre- and post-intervention

Variables	Supplement group (*n*=26)	Placebo group (*n*=25)	*P*†
Weight (kg)			
Baseline	74.55±12.20	74.40±16.10	0.97†
16 weeks	75.13±12.29	75.76±16.33	0.88†
*P**,‡	0.64	0.06	
Waist circumference (cm)			
Baseline	100.87±15.02	99.36±14.62	0.71
16 weeks	100.47±9.61	99.52±13.54	0.87
P‡	0.85	0.92	
Waist-to-hip ratio			
Baseline	0.94±0.05	0.92±0.10	0.18
16 weeks	0.95±0.56	0.92±0.11	0.18
P‡	0.02	0.01	
BMI (kg/m^2)			
Baseline	30.21±4.42	29.20±6.41	0.16
16 weeks	30.42±4.30	29.73±6.48	0.21
P¥	0.55	0.08	
25(OH) D3 (ng/mL)			
Baseline	21.32±5.85	20.73±6.47	0.81
16 weeks	29.11±11.70	22.4±11.36	0.04
P¥	<0.001**	0.89	
Energy intake (kCal/day)			
Baseline	2337.92±410.20	2203.60±442.12	0.26
16 weeks	2276.82±448.80	2084.20±257.66	0.07
P‡	0.07	0.24	
Carbohydrate intake (g/day)			
Baseline	356.29±84.41	322.25±66.19	0.11
16 weeks	343.28±73.36	311.16±56.25	0.86
P‡	0.11	0.44	
Protein intake (g/day)			
Baseline	91.80±15.57	86.99±15.07	0.33
16 weeks	86.90±17.92	100.48±9.61	0.44
P‡	0.57	0.98	
Fat intake (g/day)			
Baseline	64.29±10.61	56.25±11.51	0.02**
16 weeks	62.24±15.20	53±13.93	0.43†
P‡	0.40	0.54	
Vitamin C intake (mg/day)			
Baseline	125.66±58.79	84.72±54.17	0.02**
16 weeks	101.81±51.50	88.43±49.50	0.38
P‡	0.07	0.68	
Vitamin D intake (mcg/day)			
Baseline	0.84±1.28	0.56±0.72	0.33
16 weeks	1.06±1.23	0.77±0.79	0.33
P¥	0.48	0.27	
Saturated fatty acids intake (g/day)			
Baseline	17.89±9.16	16.11±11.24	0.27
16 weeks	16.73±8.72	12.67±6.23	0.07
P¥	0.10	0.33	
Monounsaturated fatty acids intake (g/day)			
Baseline	25.54±11.11	21.41±7.06	0.12
16 weeks	24.40±14.80	19.84±6.91	0.18
P‡	0.56	0.28	
Polyunsaturated fatty acids intake (g/day)			
Baseline	19.93±5.51	20.36±9.23	0.84
16 weeks	18.27±4.21	16.96±4.24	0.26
P‡	0.04	0.84	
Exposure to sunlight score			
Baseline	31.02±24.67	32.54±33.05	0.85
16 weeks	40.29±35.23	32.80±25.19	0.54
P‡	0.44	0.98	
Physical activity (MET/hours*day)			
Baseline	31.48±18.12	25.81±15.81	0.09
16 weeks	30.28±16.99	24.95±12.96	0.19
P¥	0.50	0.99	

Variables are represented as mean±SD. †By independent *t*-test, ‡By paired sample *t*-test, ¥By Wilcoxon signed rank test, *Indicates between groups comparisons, **P<0.05. SD=Standard deviation, BMI=Body mass index, MET=Metabolic equivalent of task

Depression score did not change significantly, however, subgroup analysis in accordance to the baseline 25-hydroxy vitamin D indicated that depression scale decreased in group with lower levels of serum vitamin D. Although recent studies indicated serum 25(OH) vitamin D concentrations have inverse relation with symptoms of depression.[32] Other studies have reported non-significant effect of vitamin D on depression in individuals with lower scores of depression,[33,34] justifying the challenge between subgroups analysis and overall result. Furthermore, vitamin D treatment for longer periods may exert therapeutic effect to ameliorate depression in patients with clinically proven depression.[35] Future randomized controlled trials are needed to examine the effect of vitamin D on patients with vitamin D deficiency and depression.

In the present study, there was no improvement in patients' stress scale. Black *et al*. in a cross-sectional study did not find any relationships between stress and serum level of vitamin D assessed through DASS-21.[12] Wang *et al*. reported that vitamin D could not reduce physiological distress in hospitalized patients with vitamin D and vitamin C deficiency.[36]

Table 3: Effects of vitamin D on inflammatory biomarkers, anxiety, stress, and depression

Variables	Intervention group (*n*=26)	Placebo group (*n*=25)	*P*	*P**
Anxiety				
Baseline	12.31±4.26	10.44±5.05	0.16†	<0.001**
16 weeks	9.15±4.54	13.28±4.61	0.02†,**	
Change	-3.15±4.65	1.64±4.75	0.001†,**	
P (within group)‡	<0.001**	0.10		
Stress				
Baseline	13.27±5.38	1.84±4.29	0.68†	0.42
16 weeks	12.2±6.63	13.28±4.61	0.51†	
Change	-1.04±4.68	-0.56±2.80	0.66†	
P (within group)‡	0.26	0.33		
Depression				
Baseline	11.88±4.84	12.44±5.09	0.69†	0.29
16 weeks	9.88±3.88	11.08±5.28	0.36†	
Change	-2.00±4.38	-1.36±4.07	0.59†	
P (within group)¥	0.03**	0.11		
hs-CRP (mg/L)				
Baseline	3.23 (1.37, 9.06)	4.12 (2.32, 10.65)	0.35£	<0.001**
16 weeks	2.39 (1.18, 7.93)	4.29 (2.56, 10.23)	0.05£	
Change	-0.27 (-0.89, -0.22)	2.58 (-0.21, 1.21)	0.01£	
P (within group)¥	0.01**	0.16		
IL-10 (ng/mL)				
Baseline	80.25 (61.75, 102.00)	93.00 (70.75, 115.00)	0.22£	0.02**
16 weeks	93.00 (64.27, 157.27)	83.00 (66.90, 106.00)	0.44£	
Change	8.80 (-2.86, 27.75)	2.1 (-11.00, 10.70)	0.04£	
P (within group)¥	0.02**	0.93		

Variables are represented mean±SD or median (25th, 75th percentiles). *By ANCOVA for between groups' comparisons (adjustment was made for baseline values, fat, and vitamin C intake), †By Independent *t*-test, ‡By paired sample *t*-test, ¥Obtained from Wilcoxon signed rank test, £Obtained from Mann-Whitney test, **$P<0.05$. SD=Standard deviation, hs-CRP=high sensitivity C-reactive protein, IL-10=interleukin 10

We found that vitamin D supplementation for 4-month period decreased hs-CRP while increased IL-10 levels. Previous observational studies have revealed that diabetic patients have significantly higher hs-CRP concentrations than healthy people.[37] Moreover, anxiety is associated with high serum levels of hs-CRP,[38] while IL-10 level, as an anti-inflammatory biomarker, is low in patients with anxiety.[7] Improving inflammatory markers in GAD could help reduce anxiety.[39] It seems that anxiety disorders are associated with defective activity of hypothalamus-pituitary-adrenal axis (HPA), higher pro-inflammatory, and low anti-inflammatory cytokines.[40,41]

Diabetic patients are at higher risk of oxidative stress, heart disease, and anxiety disorder.[42,43] Vitamin D supplementation is effective for reducing serum hs-CRP concentrations,[44] however, some authors did not report any changes in women with gestational diabetes.[45] The difference in the results of studies could be due to supplementation dosage, treatment period, and the population studied. The higher serum concentration of hs-CRP at baseline is effective in result of vitamin D interventions.[46] Supplementation with vitamin D for 35 days increased IL-10 in vitamin D insufficient subjects [serum 25(OH) D <29 ng/mL].[47] Furthermore, 3-month supplementation with 50,000 IU vitamin D increased IL-10 in multiple sclerosis patients.[48] Subgroup analysis showed that the increase in IL-10 in both subgroups with vitamin D supplementation was non-significant and could be attributed to smaller sample size in subgroups.

Vitamin D supplement had no significant effects on BMI and anthropometric indices as previous studies also confirmed these results.[49-51] A meta-analysis indicated that serum vitamin D has inverse relationship with fat mass, however, vitamin D supplementation was not effective on fat mass.[52]

Recently, it has been reported vitamin D responsive factors such as vitamin D receptors (VDRs) and the regions of serotonin receptors and tryptophan hydroxylase, have relationship with depression.[53] On the other hand, another study did not find any association between vitamin D levels, polymorphism of vitamin D receptor, and depression.[54] In the future, more studies are needed to show the mediatory role of genes in vitamin D impact.

As a limitation of our study, we were unable to measure the seasonal changes that could affect the outcome. However, the intervention was not conducted in different seasons. The strength of our study was the continuous follow-up during the intervention.

Table 4: Comparison of the baseline and post-intervention values of variables based on serum vitamin D (ng/mL) status in the study groups

Variables	10 >Vitamin D ≥20			20 >Vitamin D >30		
	Intervention group (*n*=9)	Placebo group (*n*=9)	*P*	Intervention group (*n*=17)	Placebo group (*n*=16)	*P*
Anxiety						
Baseline	12.89±3.14	11.11±5.16	0.06†	12.00±4.82	11.12±5.51	0.63†
16 weeks	9.78±3.68	10.78±4.04	0.06†	9.82±4.34	12.18±4.26	0.12†
Change	-5±4.92	2.67±2.31	0.01†,**	-2.18±3.33	1.06±2.73	0.03†,**
P (within group)‡	0.02	0.24		0.05	0.27	
Stress						
Baseline	13.22±4.23	12.77±2.63	0.79†	13.29±6.02	14.44±4.98	0.33†
16 weeks	7.88±3.89	11.89±3.69	0.96†	11.88±4.97	13.56±5.01	0.43†
Change	-0.33±3.33	-00±1.73	0.84†	0.25	0.24	0.70†
P (within group)‡	0.83	0.99		-1.42±3.91	-0.88±2.87	
Depression						
Baseline	12.77±4.14	9.22±4.15	0.46†	11.40±5.22	13.18±5.06	0.33†
16 weeks	12.89±6.27	12.78±4.02	0.68†	9.94±4.05	11.25±5.00	0.42†
Change	-3.00±2.71	-0.33±2.57	0.14†	-1.47±2.71	1.94±3.32	0.76†
P (within group)‡	0.04	0.78		0.22	0.09	
hs-CRP (mg/L)						
Baseline	2.52 (1.19, 12.19)	2.79 (1.54, 4.27)	0.73£	3.35 (1.80, 7.05)	7.18 (2.71, 12.31)	0.09£
16 weeks	2.21 (1.12, 9.09)	2.56 (1.84, 5.51)	0.07£	2.56 (1.18, 7.88)	6.01 (3.02, 11.89)	0.04£
Change	-0.75 (-1.82, -0.14)	0.35 (-0.39, 0.86)	0.01£,**	-0.21 (-0.86, 0.27)	0.23 (-1.51, 1.41)	0.08£
P (within group)¥	0.008**	0.23		0.03	0.26	
IL-10 (ng/mL)						
Baseline	68.00 (48.70, 111.20)	99.00 (59.30, 118.50)	0.34£	85.00 (62.50, 107.00)	90.00 (74.12, 116.00)	0.48£
16 weeks	92.00 (48.80, 143.25)	83.00 (63.50, 114.50)	0.76£	94.00 (81.25, 164.50)	83.50 (68.85, 109.25)	0.26£
Change	4.40 (-6.35, 19.00)	00.00 (-10.40, 6.20)	0.34£	10.00 (-3.25, 49.10)	2.55 (-20.75, 14.76)	0.08£
P (within group)¥	0.31	0.67		0.67	0.83	

Variables are represented mean±SD or median (25th, 75th percentiles). †By independent *t*-test, ‡By paired sample *t*-test, ¥Obtained from Wilcoxon signed rank test, £Obtained from Mann-Whitney test, ***P*<0.05. SD=Standard deviation, hs-CRP=high sensitivity C-reactive protein, IL-10=Interleukin 10

Conclusions

Vitamin D supplementation could improve anti-inflammatory biomarkers and reduce cardiovascular risk factor and anxiety in T2DM women with anxiety and vitamin D deficiency. More studies are warranted to clarify the beneficial effects of supplementation with vitamin D on male diabetes patients having anxiety.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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References

1. DeLuca GC, Kimball SM, Kolasinski J, Ramagopalan SV, Ebers GC. Review: The role of Vitamin D in nervous system health and disease. Neuropathol Appl Neurobiol 2013;39:458-84.
2. Holick MF. The Vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord 2017;18:153-65.
3. Kazemi A, Sharifi F, Jafari N, Mousavinasab N. High prevalence of Vitamin D deficiency among pregnant women and their newborns in an Iranian population. J Womens Health (Larchmt) 2009;18:835-9.
4. Gale C, Oakley-Browne M. Generalised anxiety disorder. Evidence Based Ment Health 2004;7:32-3.
5. Smith KJ, Béland M, Clyde M, Gariépy G, Pagé V, Badawi G, *et al.* Association of diabetes with anxiety: A systematic review and meta-analysis. J Psychosom Res 2013;74:89-99.
6. Grigsby AB, Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. Prevalence of anxiety in adults with diabetes: A systematic review. J Psychosom Res 2002;53:1053-60.
7. Furtado M, Katzman MA. Neuroinflammatory pathways in anxiety, posttraumatic stress, and obsessive compulsive disorders. Psychiatry Res 2015;229:37-48.
8. Ferreira TB, Kasahara TM, Barros PO, Vieira MM, Bittencourt VC, Hygino J, *et al.* Dopamine up-regulates Th17 phenotype from individuals with generalized anxiety disorder. J Neuroimmunol 2011;238:58-66.
9. Luis-Rodríguez D, Martínez-Castelao A, Górriz JL, De-Álvaro F, Navarro-González JF. Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. World J Diabetes 2012;3:7-18.
10. Hajebrahimi B, Kiamanesh A, Asgharnejad Farid AA,

Asadikaram G. Type 2 diabetes and mental disorders; a plausible link with inflammation. Cell Mol Biol (Noisy-le-grand) 2016;62:71-7.
11. Jhee JH, Kim H, Park S, Yun HR, Jung SY, Kee YK, *et al.* Vitamin D deficiency is significantly associated with depression in patients with chronic kidney disease. PLoS One 2017;12:e0171009.
12. Black LJ, Jacoby P, Allen KL, Trapp GS, Hart PH, Byrne SM, *et al.* Low Vitamin D levels are associated with symptoms of depression in young adult males. Aust N Z J Psychiatry 2014;48:464-71.
13. Penckofer S, Byrn M, Adams W, Emanuele MA, Mumby P, Kouba J, *et al.* Vitamin D supplementation improves mood in women with type 2 diabetes. J Diabetes Res 2017;2017:8232863.
14. Tartagni M, Cicinelli MV, Tartagni MV, Alrasheed H, Matteo M, Baldini D, *et al.* Vitamin D supplementation for premenstrual syndrome-related mood disorders in adolescents with severe hypovitaminosis D. J Pediatr Adolesc Gynecol 2016;29:357-61.
15. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37 Suppl 1:S81-90.
16. Samani S, Joukar B. A study on the reliability and validity of the short form of the depression anxiety stress scale (DASS21). Journal Of Social Science And Humanities Of Shiraz University 2007;26:65-77.
17. Henry JD, Crawford JR. The short-form version of the depression anxiety stress scales (DASS-21): Construct validity and normative data in a large non-clinical sample. Br J Clin Psychol 2005;44:227-39.
18. Asghari A, Saed F, Dibajnia P. Psychometric properties of the depression anxiety stress scales-21 (DASS-21) in a non-clinical Iranian sample. Int J Psychol 2008;2:82-102.
19. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, *et al.* Evaluation, treatment, and prevention of Vitamin D deficiency: An endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30.
20. Hejazi E, Amani R, SharafodinZadeh N, Cheraghian B. Comparison of antioxidant status and Vitamin D levels between multiple sclerosis patients and healthy matched subjects. Mult Scler Int 2014;2014:539854.
21. Webb AR. Who, what, where and when-influences on cutaneous Vitamin D synthesis. Prog Biophys Mol Biol 2006;92:17-25.
22. Fogelholm M, Malmberg J, Suni J, Santtila M, Kyröläinen H, Mäntysaari M, *et al.* International physical activity questionnaire: Validity against fitness. Med Sci Sports Exerc 2006;38:753-60.
23. Newby PK, Muller D, Hallfrisch J, Andres R, Tucker KL. Food patterns measured by factor analysis and anthropometric changes in adults. Am J Clin Nutr 2004;80:504-13.
24. Jamilian M, Foroozanfard F, Rahmani E, Talebi M, Bahmani F, Asemi Z, *et al.* Effect of two different doses of Vitamin D supplementation on metabolic profiles of insulin-resistant patients with polycystic ovary syndrome. Nutrients 2017;9. pii: E1280.
25. General Assembly of the World Medical Association. World medical association declaration of helsinki: Ethical principles for medical research involving human subjects. J Am Coll Dent 2014;81:14-8.
26. Kjærgaard M, Joakimsen R, Jorde R. Low serum 25-hydroxyvitamin D levels are associated with depression in an adult norwegian population. Psychiatry Res 2011;190:221-5.
27. Belvederi Murri M, Respino M, Masotti M, Innamorati M, Mondelli V, Pariante C, *et al.* Vitamin D and psychosis: Mini meta-analysis. Schizophr Res 2013;150:235-9.
28. Gowda U, Mutowo MP, Smith BJ, Wluka AE, Renzaho AM. Vitamin D supplementation to reduce depression in adults: Meta-analysis of randomized controlled trials. Nutrition 2015;31:421-9.
29. Bičíková M, Dušková M, Vítků J, Kalvachová B, Řípová D, Mohr P, *et al.* Vitamin D in anxiety and affective disorders. Physiol Res 2015;64 Suppl 2:S101-3.
30. Ataie-Jafari A, Qorbani M, Heshmat R, Ardalan G, Motlagh ME, Asayesh H, *et al.* The association of Vitamin D deficiency with psychiatric distress and violence behaviors in Iranian adolescents: The CASPIAN-III study. J Diabetes Metab Disord 2015;14:62.
31. Dean AJ, Bellgrove MA, Hall T, Phan WM, Eyles DW, Kvaskoff D, *et al.* Effects of Vitamin D supplementation on cognitive and emotional functioning in young adults – A randomised controlled trial. PLoS One 2011;6:e25966.
32. Kim SH, Seok H, Kim DS. Relationship between serum Vitamin D levels and symptoms of depression in stroke patients. Ann Rehabil Med 2016;40:120-5.
33. Wang Y, Liu Y, Lian Y, Li N, Liu H, Li G, *et al.* Efficacy of high-dose supplementation with oral Vitamin D3 on depressive symptoms in dialysis patients with Vitamin D3 insufficiency: A Prospective, randomized, double-blind study. J Clin Psychopharmacol 2016;36:229-35.
34. Kjærgaard M, Waterloo K, Wang CE, Almås B, Figenschau Y, Hutchinson MS, *et al.* Effect of Vitamin D supplement on depression scores in people with low levels of serum 25-hydroxyvitamin D: Nested case-control study and randomised clinical trial. Br J Psychiatry 2012;201:360-8.
35. Shaffer JA, Edmondson D, Wasson LT, Falzon L, Homma K, Ezeokoli N, *et al.* Vitamin D supplementation for depressive symptoms: A systematic review and meta-analysis of randomized controlled trials. Psychosom Med 2014;76:190-6.
36. Sepehrmanesh Z, Kolahdooz F, Abedi F, Mazroii N, Assarian A, Asemi Z, *et al.* Vitamin D supplementation affects the beck depression inventory, insulin resistance, and biomarkers of oxidative stress in patients with major depressive disorder: A randomized, controlled clinical trial. J Nutr 2016;146:243-8.
37. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, *et al.* Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern Emerg Med 2013;8:33-40.
38. Tayefi M, Shafiee M, Kazemi-Bajestani SMR, Esmaeili H, Darroudi S, Khakpouri S, *et al.* Depression and anxiety both associate with serum level of hs-CRP: A gender-stratified analysis in a population-based study. Psychoneuroendocrinology 2017;81:63-9.
39. Vieira MM, Ferreira TB, Pacheco PA, Barros PO, Almeida CR, Araújo-Lima CF, *et al.* Enhanced Th17 phenotype in individuals with generalized anxiety disorder. J Neuroimmunol 2010;229:212-8.
40. Koh KB, Lee Y. Reduced anxiety level by therapeutic interventions and cell-mediated immunity in panic disorder patients. Psychother Psychosom 2004;73:286-92.
41. Lenze EJ, Mantella RC, Shi P, Goate AM, Nowotny P, Butters MA, *et al.* Elevated cortisol in older adults with generalized anxiety disorder is reduced by treatment: A placebo-controlled evaluation of escitalopram. Am J Geriatr Psychiatry 2011;19:482-90.
42. Bertoni AG, Burke GL, Owusu JA, Carnethon MR, Vaidya D, Barr RG, *et al.* Inflammation and the incidence of type 2 diabetes: The multi-ethnic study of atherosclerosis (MESA). Diabetes Care 2010;33:804-10.
43. Thomas J, Jones G, Scarinci I, Brantley P. A descriptive and comparative study of the prevalence of depressive and anxiety

disorders in low-income adults with type 2 diabetes and other chronic illnesses. Diabetes Care 2003;26:2311-7.

44. Ngo DT, Sverdlov AL, McNeil JJ, Horowitz JD. Does Vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? Am J Med 2010;123:335-41.
45. Asemi Z, Karamali M, Esmaillzadeh A. Effects of calcium-Vitamin D co-supplementation on glycaemic control, inflammation and oxidative stress in gestational diabetes: A randomised placebo-controlled trial. Diabetologia 2014;57:1798-806.
46. Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ, *et al.* Effect of Vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: A meta-analysis of randomized controlled trials. Nutrients 2014;6:2206-16.
47. Barker T, Rogers VE, Levy M, Templeton J, Goldfine H, Schneider ED, *et al.* Supplemental Vitamin D increases serum cytokines in those with initially low 25-hydroxyvitamin D: A randomized, double blind, placebo-controlled study. Cytokine 2015;71:132-8.
48. Ashtari F, Toghianifar N, Zarkesh-Esfahani SH, Mansourian M. Short-term effect of high-dose Vitamin D on the level of interleukin 10 in patients with multiple sclerosis: A randomized, double-blind, placebo-controlled clinical trial. Neuroimmunomodulation 2015;22:400-4.
49. Salekzamani S, Mehralizadeh H, Ghezel A, Salekzamani Y, Jafarabadi MA, Bavil AS, *et al.* Effect of high-dose Vitamin D supplementation on cardiometabolic risk factors in subjects with metabolic syndrome: A randomized controlled double-blind clinical trial. J Endocrinol Invest 2016;39:1303-13.
50. Zittermann A, Frisch S, Berthold HK, Götting C, Kuhn J, Kleesiek K, *et al.* Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. Am J Clin Nutr 2009;89:1321-7.
51. Sharifi N, Amani R, Hajiani E, Cheraghian B. Women may respond different from men to Vitamin D supplementation regarding cardiometabolic biomarkers. Exp Biol Med (Maywood) 2016;241:830-8.
52. Golzarand M, Hollis BW, Mirmiran P, Wagner CL, Shab-Bidar S. Vitamin D supplementation and body fat mass: A systematic review and meta-analysis. European Journal of Clinical Nutrition. 2018. [In press].
53. Wang TT, Tavera-Mendoza LE, Laperriere D, Libby E, MacLeod NB, Nagai Y, *et al.* Large-scale *in silico* and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol 2005;19:2685-95.
54. Can MŞ, Baykan H, Baykan Ö, Erensoy N, Karlıdere T. Vitamin D levels and Vitamin D receptor gene polymorphism in major depression. Psychiatr Danub 2017;29:179-85.