# Exploring the Effects of Vitamin D Supplementation on Cognitive Functions and Mental Health Status in Subjects Under Methadone Maintenance Treatment

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**Objectives:** Vitamin D deficiency may be linked to several mental complications including cognitive deficits, depression, and anxiety in patients under methadone maintenance treatment (MMT). This study was designed to explore the effect of vitamin D supplementation on cognitive functions and mental health parameters in subjects under MMT.

**Methods:** This randomized, double-blinded, placebo-controlled clinical trial was carried out among 64 patients under MMT. Participants were randomly allocated to receive either 50,000 IU vitamin D supplements (n = 32) or placebo (n = 32) every 2 weeks for 24 weeks. Cognitive functions and mental health parameters were taken at baseline and posttreatment to evaluate relevant variables.

**Results:** After the 24-week intervention, compared with the placebo, serum 25(OH) vitamin D levels significantly increased in participants who received vitamin D supplements (β 14.50; 95% confidence interval [CI], 13.17–15.83; $P < 0.001$). In addition, compared with the placebo, subjects who received vitamin D had a significant reduction in Iowa Gambling Task (β −6.25; 95% CI, −8.60 to −3.90; $P < 0.001$), and significant increases in Verbal Fluency Test (β 2.82; 95% CI, 0.78–4.86; $P = 0.007$), Immediate Logic Memory (β 1. 32; 95% CI, 0.27–2.37; $P = 0.01$), Reverse Digit Span (β 2.06; 95% CI, 1.18–2.94; $P < 0.001$) and visual working memory (β 0.75; 95% CI, 0.33–1.16; $P = 0.001$). Also, vitamin D supplementation significantly improved BDI (β −2.76; 95% CI, −3.97 to −1.55; $P < 0.001$) compared with the placebo. When we applied Bonferroni correction, LM-Immediate ($P = 0.07$) became nonsignificant, and other mental health parameters did not alter.

**Conclusions:** Overall, taking 50,000 IU vitamin D supplements every 2 weeks for 24 weeks by patients under MMT had beneficial effects on cognitive functions and some mental health parameters. Further studies are needed to confirm our findings.

**Key Words:** cognitive functions, mental health, methadone maintenance treatment, vitamin D supplementation

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Methadone maintenance treatment (MMT) is suggested for treating opioid use disorder (Kourounis et al., 2016). In Iran, the prevalence of opioid use is rising and was nearly 3 times higher than the prevalence worldwide. About 1.2 million Iranians have opioid dependency with opium as the most commonly used opioid (82.3%), followed by opium ashes (as it was boiled down to be prepared for use) (27.8%), methadone for non-medical usages (16.6%), heroin and heroin/cracked (crack heroin is the crystal form of heroin) (16.1%), and morphine (2.6%) (Amin-Esmaeili et al., 2016). The percentages add up to more than 100% because some subjects used more than 1 type of opioid. About 500,000 individuals are under methadone and buprenorphine maintenance treatments (Danial et al., 2014). In a study by Darke et al. (2000), it was reported higher cognitive deficits in a controlled study (30 subjects and 30 controls). This finding was replicated by Mazhari et al. (2015). Previous studies have shown that MMT influences mental health parameters, such as sleep, anxiety, and depression disturbances (Callaly et al., 2001; Fan et al., 2014). Earlier, in a cross-sectional study conducted by Kim et al. (2009), low vitamin D status was identified among 52% of subjects with MMT. They also highlighted that this finding is nonspecific to MMT yet common among debilitated populations (Kim et al., 2009). Data representing the circulating vitamin D status in patients

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with MMT from other countries is scarce. Hypovitaminosis D might be linked to impaired cognitive functions (Llewellyn et al., 2009), periodontal disease and tooth loss (Dietrich et al., 2004), and other mental health disorders (Nerhus et al., 2015).

Recently, vitamin D administration was suggested in patients under MMT (Ghaderi et al., 2017a,b). This may be because of the beneficial effects of vitamin D intake on diseases linked to mental health disorders in these patients. We have previously documented that consuming 50,000 IU vitamin D every 2 weeks for 12 weeks in individuals under MMT had favorable effects on mental health parameters including depression, anxiety, and sleep disorder (Ghaderi et al., 2017a,b). Current evidence has reported the controversial effects of vitamin D supplementation on mental health parameters and cognitive functions in patients not undergoing MMT. We have previously demonstrated that vitamin D administration at a dosage of 50,000 IU/week for 8 weeks to patients with major depressive disorder had beneficial effects on the Beck Depression Inventory (BDI) (Sepehrmanesh et al., 2016). However, a meta-analysis study by Gowda et al. (2015) did not show any significant reduction in depression score following vitamin D supplementation. Moreover, 2 other meta-analyses (Li et al., 2014; Shaffer et al., 2014) of randomized controlled trials, evaluating the efficacy of vitamin D supplementation on depression in adults, did not reveal any significant effect on depressive symptoms. Also, joint supplementation with vitamin D3 (400 IU) and calcium (1000 mg) did not affect the treatment of cognitive impairment in elderly women (Rossom et al., 2012). Moreover, vitamin D supplementation (50,000 IU/week) for 52 weeks to persons undergoing dialysis did not reduce depressive symptoms (Wang et al., 2016). Excess amount of vitamin D might cause hypercalcemia, which is a strong indication of vitamin D toxicity along with an increase in urination and thirst (Koul et al., 2011). Left untreated, hypercalcemia might result in extra deposits of calcium in soft tissues and organs. such as the kidneys, liver, and heart, resulting in pain and organ damage (Koul et al., 2011).

Vitamin D may improve mental health status through regulating the synthesis of serotonin (5- hydroxyltryptamine) in brain (Stockmeier, 2003). In addition, vitamin D might improve parameters of mental health through increasing the expression of neurotrophic factors, the stimulation of adult neurogenesis, and the regulation of calcium homeostasis (Garcion et al., 2002; Bourre, 2006; McCann and Ames, 2008). McCann and Ames (2008) concluded that the current experiential evidence does not yet fully satisfy causal criteria for cognitive functions. They nevertheless supported supplementation as ''prudent'' among debilitated populations (McCann and Ames, 2008). Numerus studies demonstrated that MMT is linked to impaired cognitive functions and this impairment included a range of mental function domains, such as phonemic word fluency, psychomotor performance, speed of processing, such as Trial Making Test (TMT), attention, long-term and short-term memory scales, such as Digit Span (DGSP), and decision-making process, such as Iowa Gambling Task (IGT) (Darke et al., 2000; Specka et al., 2000, Mintzer and Stitzer, 2002; Mazhari et al., 2015). On the other hand, previous studies have documented that vitamin D may influence brain and neuron development as well as have beneficial effects on cognitive functions, such as speed of processing, memory function, and decision-making in patients without MMT (Soni et al., 2012; Annweiler et al., 2013). To our best knowledge, data on the effects of vitamin D supplementation on cognitive function improvement in patients under MMT are limited. Therefore, we assumed that the combination therapy of vitamin D and methadone in opioid withdrawal protocols could be introduced for increasing the quality of life and decreasing the MMT-related side effects. The aim of the current study was to investigate the effects of vitamin D supplementation on cognitive functions and mental health status in subjects under MMT.

## METHODS

### Trial Design and Participants

This randomized, double-blinded, placebo-controlled clinical trial was registered in the Iranian website for registration of clinical trials at http://www.irct.ir: IRCT2017101133079N4, the Primary Registry in the WHO Registry Network set up in collaboration with Ministry of Health and Medical Education. This study was conducted among 64 men under MMT, ages 18 to 60 years who were referred to the Golabchi Clinic in Kashan, Iran, from October 2017 to March 2018. Study protocol was approved by the research ethics committee of Kashan University of Medical Sciences (KAUMS). This investigation was conducted in accordance with the Declaration of Helsinki and informed consent was signed by all participants. All informed consent forms were reviewed by the research ethics committee of KAUMS. Exclusion criteria were not living in Kashan, taking vitamin D, multivitamin-mineral, and antioxidant supplements during the last 3 months before the intervention.

### Study Design

At the onset of the study, to avoid potential confounding effects, all participants were stratified randomized according to age and BMI. Then, participants in each block were randomly allocated into 2 treatment groups to take either 50,000 IU vitamin D (Zahravi, Tabriz, Iran) or placebo (Zahravi, Tabriz, Iran) ($n = 32$ each group) every 2 weeks for 24 weeks. The placebos were matched in terms of their colour, shape, size, packaging, smell, and taste with the vitamin D3 capsules. We used the above-mentioned dose of vitamin D based on a prior study published in subjects with chronic liver diseases (Stokes et al., 2016). Methadone was consumed in the form of syrup by patients. To evaluate the compliance rate, participants' serum 25(OH) vitamin D levels were measured at weeks 0, 8, 16, and 24 of the intervention using an enzyme-linked immunosorbent assay (ELISA) method. All patients completed a 3-day food record and 3 physical activity records as metabolic equivalents (METs) (Ainsworth et al., 2000) at weeks 0, 6, 12, 18, and 24 of the intervention. To calculate participants' nutrient intakes using 3-day food records (Sciences, 2002), we applied Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian food pattern. Previous studies have reported the impact of dietary intakes and physical activity

on cognitive functions and mood state (Ortega et al., 1997; Ruscheweyh et al., 2011; Loprinzi and Kane, 2015), therefore, we used food record and physical activity questionnaire in order to incorporate dietary intakes and physical activity as the confounders in this study.

## Anthropometric Measures

Patients' anthropometric measurements were conducted using a standard scale (Seca, Hamburg, Germany) at baseline and 24 weeks after supplementation. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in square meters . Previous studies exploring the relationship between indices of central adiposity and cognitive functions are based on being older adults (65+) (Driscoll et al., 2011), cross-sectional studies (Nourhashemi et al., 2002), or using small sample sizes (Yoon et al., 2012). Therefore, we recorded participants' anthropometric measures to be used as the confounder in this study.

## Outcomes

Cognitive functions, including TMT, IGT, Wechsler Memory Scale (WMS), Scored General Intelligence Test (SGIT), and DGSP backwards in Wechsler intelligence Scale were considered as the outcomes of interest and BDI and Beck Anxiety Inventory (BAI) were considered as the secondary outcomes.

## Clinical Assessment

TMT is normally administered using 2 sub-components, which are known as TMT-A and TMT-B (Stuss et al., 2001). TMT measures a variety of cognitive functions including attention, visual search and scanning, sequencing and shifting, psychomotor speed, abstraction, and cognitive flexibility (Salthouse, 2011). TMT is normally administered using 2 sub-components, which are known as TMT-A and TMT-B. In TMT-A, the patient is presented with encircled numbers from 1 to 25 randomly distributed on a sheet of paper, and they are instructed to link the numbers in ascending order (ie, 1-2-3. . .) using a pen or pencil. In TMT-B, a second sheet includes both encircled numbers and letters that the patient must link in alternating ascending order (ie, 1-A-2-B-. . .). Task performance in each part is typically quantified by measuring the completion time, with TMT-B taking longer to complete. IGT is a useful tool for analyzing the individuals' decision-making process. The participants were faced with 4 cards (Businelle et al., 2008; Turnbull et al., 2014). Memory scale was measured by Logical Memory (I and II). It is a subtest of Wechsler Memory Scale (WMS), assessing the narrative memory under a free recall condition (Wechsler, 1997). Scored General Intelligence Test (SGIT) is one of the few tests that attempts to measure general intelligence and can be administered by the clinician during the psychiatric interview (Canivez et al., 2017). Short-term auditory memory was measured by DGSP backwards in Wechsler Memory Scale-III (WMS- III) (Wechsler, 1997). The examiner read a list of 3 to 9 digits calmly and loudly, and the participant should read them in the same way after listening to each list. DGSP is normally administered as 2 sub-components known as DGSP-Straight and DGSP-Reverse (Jasinski et al., 2011). The FAS Test, a subtest of the Neurosensory Center Comprehensive Examination for Aphasia (Crockett, 1977) is a measure of phonemic word fluency. BDI was assessed using a self-compiled questionnaire (Beck et al., 1961). BDI was assessed using a self-compiled questionnaire (Beck et al., 1961). The internal stability of the test among Iranian students (Persian version of BDI-II) was moderate-to-good (Cronbach $\alpha = 0.58$) and its reliability by test-retest was 0.73 (Meygoni and Ahadi, 2012). Anxiety was measured using BAI-21, which was developed by Beck et al. (1988) to determine the frequency of anxiety symptoms in adults. Kaviani and Mousavi (2008) approved the validity and reliability of Persian version of BAI among Iranian normal population as well as clinically anxious patients.

## Biochemical Assessment

At baseline and week 24 of the treatment, 10 mL fasting blood samples were collected from each patient at Kashan reference laboratory. Serum 25-hydroxyvitamin D values were measured using a commercial ELISA kit (IDS, Boldon, UK) with intra- and inter-assay coefficient variances (CVs) of lower than 7%.

## Sample Size

Sample size was calculated using the formula suggested for randomized clinical trials. Type 1 ($\alpha$) and type 2 errors ($\beta$) were defined as 0.05, and 0.20 to have the study power of 80%. We did not find a similar study regarding the effects of vitamin D on primary outcomes of this study for determining sample size; therefore, the sample size was calculated based on the effects of vitamin D supplementation on BDI. On the basis of a previous published study (Sepehrmanesh et al., 2016), we used 6.6 as the effect size (the mean difference) of the BDI and 8.9 as SD. So, 30 participants were required in each treatment group. Considering 20% dropouts in each group, the final sample size was 35 participants in each intervention group.

We used the standard deviation of the BDI from the Sepehrmanesh et al. (2016) article (8.9) with similar study design to ours. For sample size calculation, the minimal clinically important effect size is also required, which is determined by the researcher (it should not be derived from the literature). In addition, we hypothesized that the effect size of 6.6 for BDI would result in a significant change in BDI of patients under MMT. Also the standardized effect size equals $6.6/8.9 = 0.74$, which is considered as a large effect size according to Cohen (Mansournia and Altman 2018). Using SD of 8.9, we have at least 80% power (probability) of detecting a difference of equal to or greater than 6.6 (if it really exists) as statistically significant at the 5% level.

## Randomization

Randomization was conducted using computer-generated random numbers. Randomization and allocation were concealed from the researchers and patients until the completion of final analyses. The randomized allocation sequence, enrolling patients and allocating them into intervention groups were performed by a trained staff at the clinic. Another staff, who was not involved in the trial and not aware of

random sequences, assigned the subjects to the numbered bottles of capsules.

## Statistical Analysis

To test the normality of variables, Kolmogorov-Smirnov test was used. Anthropometric and dietary characteristics of the intervention groups were compared using independent sample *t*-test. Multiple linear regression models were used to assess treatment effects on study outcomes after adjusting for confounding variables including the baseline values of outcomes as well as age and BMI at baseline. The effect sizes were presented as the mean differences with 95% confidence intervals (CIs). Bootstrapping was also used as a sensitivity analysis of CIs and inverse probability weighting was used to account for loss-to-follow-up, but the results did not change substantially. Bonferroni correction (ie, multiplying *P*-values by the number of tests) was applied to account for multiple outcome testing. The *P*-values of $<0.05/7$ or 0.007 were considered statistically significant. All statistical analyses used the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL).

# RESULTS

Three individuals in the treatment group [(because of moving to other city (n = 1) and not interested to be part of research anymore (n = 2)] and 3 in the placebo group [(because of moving to other city (n = 2) and not interested in research (n = 1)] withdrew from the trial because of personal reasons. Finally, 64 participants [vitamin D (n = 32) and placebo (n = 32)] completed the study (Fig. 1). The compliance rate

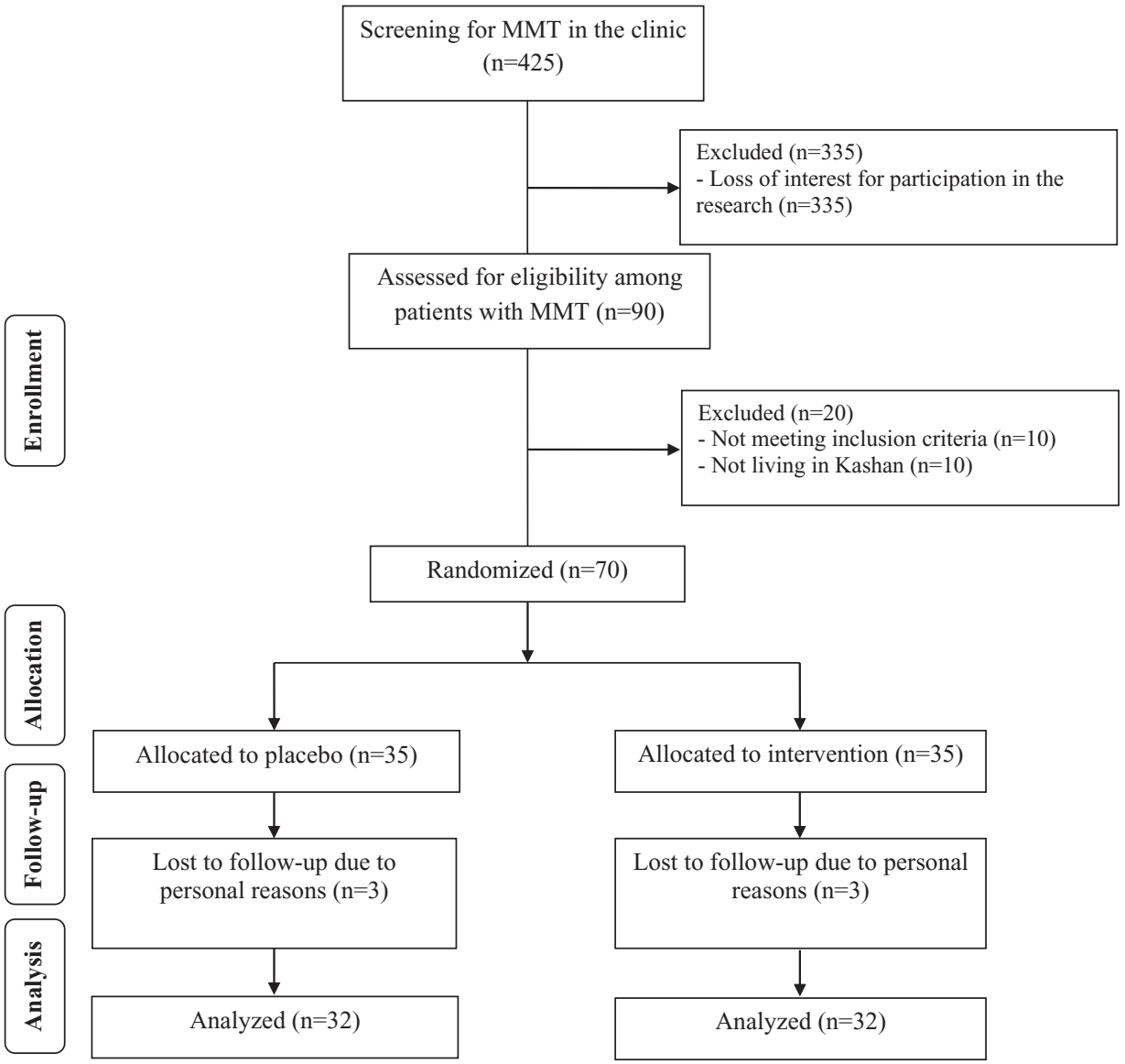


**FIGURE 1.** Summary of patient flow.

**TABLE 1.** General Characteristics of the Study Participants*

| | Placebo Group (n = 32) | Vitamin D Group (n = 32) |
|---|---|---|
| Age, y | 40.8 ± 9.5 | 37.5 ± 10.8 |
| Height, cm | 170 ± 6.6 | 169.0 ± 9.1 |
| Weight at study baseline, kg | 74.8 ± 11.6 | 71.2 ± 12.3 |
| Weight at the end-of-trial, kg | 75 ± 10.8 | 71.3 ± 11.5 |
| Weight change, kg | −0.15 ± 1.5 | −0.03 ± 1.6 |
| BMI at study baseline, kg/m$^2$ | 25.84 ± 3.5 | 24.94 ± 4.0 |
| BMI at the end-of-trial, kg/m$^2$ | 25.91 ± 3.3 | 24.96 ± 3.7 |
| BMI change, kg/m$^2$ | 0.07 ± 0.58 | −0.02 ± 0.55 |
| SGIT | 33.5 ± 3.1 | 32.9 ± 3.4 |

*Data are mean ± SDs.
SGIT, Scored General Intelligence Test.

in our study was high; more than 90% of capsules were taken during the trial in both intervention groups. To determine the compliance rate, the remaining capsules were counted and subtracted from the total amount of supplements provided to the participants. There were no adverse reactions reported by participants following the consumption of vitamin D in patients with MMT.

Mean age and anthropometric measures including height, weight, SGIT (IQ) and BMI at both baseline and end-of-trial were not significantly different between vitamin D and placebo groups (Table 1).

Macro- and micronutrient intakes, calculated using a 3-day food record, were not significantly different between 2 treatment groups (data not shown).

After the 24-week intervention, compared with the placebo group, serum 25(OH) vitamin D levels significantly increased in patients receiving vitamin D supplements (β 14.50; 95% CI, 13.17, 15.83; $P < 0.001$) (Table 2). In addition, compared with the placebo, subjects who received vitamin D had a significant reduction in IGT (β −6.25; 95% CI, −8.60 to −3.90; $P < 0.001$), and significant increases in Verbal Fluency Test (β 2.82; 95% CI, 0.78–4.86; $P = 0.007$), LM-Immediate (β 1. 32; 95% CI, 0.27–2.37; $P = 0.01$), DGSP-Reverse (β 2.06; 95% CI, 1.18–2.94; $P < 0.001$) and visual working memory (β 0.75; 95% CI, 0.33–1.16; $P = 0.001$). Also, vitamin D supplementation significantly improved BDI (β −2.76; 95% CI, −3.97 to −1.55; $P < 0.001$) compared with the placebo. There was no significant effect of vitamin D administration on measured cognitive functions and mental health parameters. When we applied Bonferroni correction, LM-Immediate ($P = 0.07$) became nonsignificant, and other mental health parameters did not alter.

**TABLE 2.** The Effect of Vitamin D Supplementation on Cognitive Functions and Mental Health Parameters in Methadone Maintenance Treatment Patients

| Variables | Placebo Group (n = 32) | | Vitamin D Group (n = 32) | | Difference in Outcome Measures Between Vitamin D and Placebo Treatment Groups* | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Week 24 | Baseline | Week 24 | β (95% CI) | $P^\dagger$ | $P^\ddagger$ |
| 25-OH-vitamin D (ng/mL) | 13.1 ± 3.6 | 12.0 ± 4.1 | 14.1 ± 4.2 | 27.5 ± 4.2 | 14.50 (13.17,15.83) | <0.001 | <0.001 |
| IGT | 28.2 ± 4.5 | 29.2 ± 3.5 | 29.3 ± 3.6 | 23.1 ± 3.1 | −6.25 (−8.60, −3.90) | <0.001 | <0.001 |
| TMT subscales | | | | | | | |
| TMT-A | 21.0 ± 2.6 | 20.7 ± 2.9 | 20.9 ± 2.4 | 20.7 ± 1.7 | −0.11 (−1.38, 1.16) | 0.86 | >0.99 |
| TMT-B | 54.3 ± 7 | 51.8 ± 4.9 | 52.7 ± 7.7 | 50.5 ± 4.8 | −0.91 (−3.52, 1.70) | 0.48 | >0.99 |
| Verbal Fluency Test (FAS test) | | | | | | | |
| Numbers of total words | 35.3 ± 2.6 | 35.4 ± 3.3 | 36.7 ± 3.2 | 38.6 ± 4.2 | 2.82 (0.78, 4.86) | 0.007 | 0.04 |
| LM subscales | | | | | | | |
| LM-Immediate | 28.2 ± 3.5 | 30.38 ± 2.4 | 28.9 ± 3.8 | 32.2 ± 2.1 | 1.32 (0.27, 2.37) | 0.01 | 0.07 |
| LM-Delayed | 9.5 ± 1.5 | 10.6 ± 1.1 | 10.1 ± 1.4 | 10.9 ± 1.2 | 1.49 (0.33, 2.66) | 0.33 | >0.99 |
| DGSP | | | | | | | |
| DGSP-Straight | 9.1 ± 1.9 | 10.6 ± 1.7 | 9.0 ± 2 | 10.1 ± 1.5 | −0.56 (−1.39, 0.26) | 0.17 | >0.99 |
| DGSP-Reverse | 8.7 ± 1.6 | 7.4 ± 1.7 | 8.8 ± 1.4 | 9.3 ± 1.6 | 2.06 (1.18, 2.94) | <0.001 | <0.001 |
| Visual working memory | 3.53 ± 0.91 | 4.02 ± 0.52 | 3.99 ± 0.94 | 4.88 ± 1.05 | 0.75 (0.33, 1.16) | 0.001 | 0.007 |
| BAI | 15.1 ± 2.7 | 17.8 ± 5.2 | 16.7 ± 4.8 | 19.0 ± 3.7 | 1.29 (−1.02, 3.61) | 0.26 | >0.99 |
| BDI | 15.9 ± 4.4 | 16.1 ± 2.9 | 14.8 ± 5.6 | 12.7 ± 4.6 | −2.76 (−3.97, −1.55) | <0.001 | <0.001 |

Data are mean ± SDs.
*"Outcome measures" refers to the change in values of measures of interest between baseline and week 24. β [difference in the mean outcomes measures between treatment groups (Vitamin D group = 1 and placebo group = 0)].
†Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age and baseline weight).
‡Obtained from multiple regression model and corrected using Bonferroni correction (*P*-value*7).
BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; CI, confidence interval; DGSP, Digit Span; DGSP-Reverse, Reverse Digit Span; DGSP-Straight, Straight Digit Span; IGT, Iowa Gambling Task; LM, Logic Memory; LM-Delayed, Delayed Logic Memory; LM-immediate, Immediate Logic Memory; TMT, Trial Making Test; TMT-A, Trial Making Test-form A; TMT-B, Trial Making Test-form B.

## DISCUSSION

We evaluated the effects of vitamin D supplementation on mental health status and cognitive functions after 24 weeks in subjects under MMT. Our study evidenced that taking vitamin D supplements for 24 weeks by subjects under MMT, compared with the placebo, improved BDI, IGT, FAS, LM-Immediate, DGSP-Reverse, and visual working memory, but did not affect BAI, TMT subscales, LM-Delayed and DGSP-Straight. When we applied Bonferroni correction for multiple outcome testing, LM-Immediate became nonsignificant, and other mental health parameters did not alter. Previous studies have reported that hypovitaminosis D and low BMD were present in a majority of subjects recruited from an MMT program (Kim et al., 2006, 2009). On the basis of these findings, vitamin D may be an appropriate adjunct therapy for opioid-dependent patients under treatment with MMT. It must be kept in mind that in the vitamin D group, increased levels of serum 25(OH) vitamin D were statistically significant, yet not clinically significant. Long-term interventions and higher dosage of vitamin D might be required to provide greater changes in serum 25(OH) vitamin D levels.

### Effects on Mental Health

We found that vitamin D administration to patients under MMT for 24 weeks improved depression indexes, but did not affect anxiety scores. Several studies have investigated the effects of vitamin D supplementation on mental health parameters in participants with and without MMT, but the results are controversial. Ghaderi et al. (2017) observed that vitamin D supplementation (50,000 IU/week) for 12 weeks improved Pittsburgh Sleep Quality Index and BDI in patients under MMT. Huang et al. (2013) also found that vitamin D supplementation at a dosage of 50,000 IU/week improved various aspects of quality of life in veterans with multiple areas of chronic pain. We specified 2 trials demonstrating a significant effect of vitamin D intake on depression and anxiety symptoms (Lansdowne and Provost, 1998; Jorde et al., 2008), although no considerable effect was observed by others (Vieth et al., 2004; Sanders et al., 2011). In another study, vitamin D supplementation (40,000 IU/week) for 6 months had no significant effect on depression scores (Kjaergaard et al., 2012). The accurate mechanism of vitamin D in the brain and its effects on mental health parameters is not completely understood. Increased expression of tyrosine hydroxylase gene and promoted dopamine, noradrenalin, and adrenalin bioavailability might clarify the beneficial effects of vitamin D on mental health parameters (Humble, 2010; Khoraminya et al., 2013).

### Effects on Cognitive Functions

Our study demonstrated that consuming vitamin D supplements for 24 weeks by patients under MMT significantly improved IGT, FAS, LM-Immediate, DGSP-Reverse, and visual working memory, but did not affect TMT subscales, LM-Delayed and DGSP-Straight. When we applied Bonferroni correction for multiple outcome testing, LM-Immediate became nonsignificant, and other mental health parameters did not alter. Although data presenting the effects of vitamin D supplementation on cognitive functions in MMT subjects are scarce, several studies have evaluated the effects of vitamin D supplementation on cognitive functions in participants not taking MMT. In a meta-analysis by Annweiler et al. (2013), higher vitamin D concentrations were correlated with better working memory performance. Also, Assmann et al. (2015) found that higher 25(OH) D concentrations were linked to a better working memory. Furthermore, receiving vitamin D (200 IU/day) was linked to lower mean cognitive functions scores (Annweiler et al., 2010b). However, no evidence showed the association between vitamin D levels and cognitive disturbance among elder women (Annweiler et al., 2010a). In a meta-analysis by Bolland et al. (2010), vitamin D intakes were linked to a 30% increased risk of myocardial infarction, stroke, and with small yet nonsignificant increases in cognitive impairment. Moreover, Dean et al. (2011) observed that vitamin D supplementation at a dosage of 5000 IU/week for 6 months did not influence cognitive or emotional functioning in healthy young adults. In the current study, we did not find any significant effect of vitamin D supplementation on TMT-A, TMT-B, LM-Delayed, DGSP-Straight, and BAI. The controversial findings might be explained through different study designs, baseline values of measured cognitive functions and mental health parameters, baseline levels of 25-OH-vitamin D, different dosages and type of vitamin D used, and the duration of supplementation as well as different participants' characteristics. In order to improve some of the cognitive functions and mental health parameters like TMT-A, TMT-B, LM-Delayed, DGSP-Straight, and BAI, individuals might need higher concentrations of 25-OH-vitamin D. Other parameters including participants' characteristics like cognitive functions and mental health parameters, higher doses of vitamin D or longer intervention might be required to provide appropriate circulating levels of 25-OH-vitamin D necessary for improving TMT-A, TMT-B, LM-Delayed, DGSP-Straight, and BAI. Therefore, further studies were required to confirm our findings. The impact of vitamin D on IGT, FAS, LM-Immediate, DGSP-Reverse, and visual working memory also might be related to the excitatory effects of vitamin D on acetylcholine release (Izquierdo, 1990). Acetylcholine is an important neurotransmitter for learning and memory consolidation, and elevated levels of acetylcholine result in improved memory.

There were some limitations in this study. In the current study, we did not specify vitamin D intake through sun exposure. This should be considered in the interpretation of our findings as one of the limitation of this study. In addition, we did not evaluate biomarkers of inflammation and oxidative stress in the current study. In the current study, cognitive functions, including TMT, IGT, WMS, SGIT, and DGSP were considered as the primary outcomes. We did not find a similar study regarding the effects of vitamin D on primary outcomes of this study for determining sample size; therefore, the sample size was calculated based on one of the secondary outcomes (BDI). As the study is not powered to detect differences in primary outcome measures, one cannot make any definite conclusion about them. Therefore, further large-scale studies are required to examine the effect of vitamin D supplementation on cognitive functions and mental health

status in subjects under MMT by considering sample size calculation based on the primary outcomes.

## CONCLUSIONS

Overall, we found that taking vitamin D supplements for 24 weeks by subjects under MMT improved BDI, IGT, FAS, LM-Immediate, DGSP-Reverse, and visual working memory, compared with the placebo, yet did not affect BAI, TMT subscales, LM-Delayed, and DGSP-Straight. Further studies are needed to show the relative impact of vitamin D supplementation on a debilitated population versus MMT and perhaps larger samples should be used to study the parameters of vitamin D supplementation as a public health measure. Furthermore, additional studies are required to look at the functional improvement in patients under MMT following vitamin D supplementation and other micronutrients.

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