Research Paper

Vitamin D Supplementation in Chronic Schizophrenia Patients Treated with Clozapine: A Randomized, Double-Blind, Placebo-controlled Clinical Trial

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ABSTRACT

Background: While accumulating evidence suggests that vitamin D deficiency may be involved in the risk to develop schizophrenia and its outcome, there are no studies on vitamin D supplementation in this context. We sought to assess the effect of vitamin D supplementation on psychiatric, cognitive and metabolic parameters in chronic clozapine-treated schizophrenia patients.

Methods: This eight-week, randomized, double-blind, placebo-controlled clinical trial, recruited schizophrenia patients who had been maintained on clozapine treatment for at least 18 weeks and had low levels of vitamin D (<75 nmol/l) and total PANSS scores >70 (to ascertain the presence of residual symptoms). Patients were randomly allocated to either weekly oral drops of vitamin D (14,000 IU) or placebo and subsequently assessed at two-week intervals for psychosis severity, mood, cognition and metabolic profile.

Results: Twenty four patients were randomly assigned to vitamin D (aged 39.4 ± 9.6 years, 75% males) and the other 23 patients to the placebo arm (aged 42.5 ± 11.2 years, 60.9% males). After eight weeks, the vitamin D group exhibited a significant increase in vitamin D levels (31.4 vs -0.4 nmol/l, $p < 0.0001$). There was no significant effect of vitamin D on psychotic, depressive or metabolic parameters. However, in the vitamin D group, there was a trend towards improved cognition (effect size = 0.17, significance lost following Bonferroni correction).

Conclusions: Vitamin D supplementation was associated with a trend towards improved cognition, but did not affect psychosis, mood or metabolic status. It is possible that the robust decrease in the PANSS scores in both groups may have obscured an effect of vitamin D supplementation.

1. Introduction

Chronic schizophrenia patients maintained on clozapine represent the most difficult-to-treat portion of this population (Kane, 1996; Meltzer, 1997). Clozapine treatment is indicated for patients who do not respond sufficiently to numerous other antipsychotic treatments (Suzuki et al., 2011). Still, unfortunately, about 50% of patients receiving clozapine remain with significant residual symptoms despite attempts at optimization of treatment and augmentation with other psychotropic drugs (Buckley et al., 2001). Moreover, there is no consensus on effective augmentation of clozapine treatment (Lieberman and Stroup, 2011; Porcelli et al., 2011). Besides reducing psychotic burden, ameliorating cognitive impairments and negative symptoms is an important challenge for this population of schizophrenia patients. Impairment of executive functions and social cognition is one of the major disabilities in schizophrenia (Green and Harvey, 2014; McCleery et al., 2014).

The prevalence of physical health comorbidities is high in schizophrenia patients (Correll et al., 2017; De et al., 2011; Vancampfort et al., 2016) and consists mainly of metabolic syndrome(De et al., 2009c; Gardner-Sood et al., 2015; Vancampfort et al., 2015) that leads to a higher rate of cardiovascular morbidity(Correll et al., 2017;De et al.,

2009a; Vancampfort et al., 2013). Cardio-metabolic complications are associated with significantly higher mortality rates and reduced life expectancy in the range of 15–20 years, compared to non-schizophrenia populations (De et al., 2009b; Hjorthoj et al., 2017; Nielsen et al., 2013). Recently it was shown that low vitamin D levels are associated with increased risk for metabolic syndrome and cardiovascular illness in schizophrenia patients (Lally et al., 2016).

The body content of 25 (OH) vitamin D is dependent on its synthesis in the skin by the enzyme 25-hydroxylase and its consumption in the diet. Increasing evidence suggests that vitamin D is important to brain development (Cui et al., 2013; Eyles et al., 2003; Groves et al., 2013). The vitamin D receptor and the enzyme 1-α-hydroxylase that is needed for the hydroxylation of the precursor molecule 25(OH) vitamin D to the active form, have widespread expression in the adult human brain (Eyles et al., 2013). Animal models of rats deprived of vitamin D in-utero showed enhanced spontaneous hyperlocomotion and increased activity on the elevated plus maze as well as impaired latent inhibition, which is a prominent feature of schizophrenia and attributable to impaired sensory gating and attentional deficits (Burne et al., 2014; Eyles et al., 2009). Epidemiological studies found ecological factors associated with increased risk for developing schizophrenia, such as latitude, urbanicity and dark skin tone, factors that are associated also with hypovitaminosis D (McGrath et al., 2010a). Previous studies also showed that low blood vitamin D levels during the first year of life increase the risk for psychosis in males (McGrath et al., 2010b). Additionally, infants who did not receive sufficient vitamin D supplements were also found to be at risk to develop psychosis later in adulthood (McGrath et al., 2004). Furthermore, several cross-sectional studies found high prevalence of vitamin D deficiency in schizophrenia patients (Boerman et al., 2016; Itzhaky et al., 2012) and two meta-analyses concluded that the vitamin D levels of schizophrenia patients tend to be low, compared to healthy controls (Belvederi et al., 2013; Valipour et al., 2014). Vitamin D deficiency has also been suggested to be associated with cognitive impairment in people with psychotic disorders (Nerhus et al., 2017).

However, the authors were unable to find any study that evaluated the direct effect of vitamin D supplementation on psychiatric, cognitive and metabolic status in schizophrenia patients and particularly in clozapine-treated patients. This is specifically important for this population since there are limited further treatment options for clozapine-treated patients with residual symptoms. We hypothesized that Vitamin D supplementation may serve as a relatively safe adjunctive treatment for schizophrenia patients with a partial response to clozapine. We assumed that increasing vitamin D levels in clozapine-treated chronic schizophrenia patients may lead to a corresponding improvement in their psychotic, cognitive, affective and metabolic parameters.

2. Materials and Methods

2.1. Participants

All inpatients and outpatients treated with clozapine during the period between 1.5.2014 and 30.9.2016 at Geha Mental Health Center (GMHC) were identified using the electronic medical record registry ($N = 174$). Potential participants were approached by the study nurse regarding participation in the study. The study was conducted according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (see CONSORT diagram – Fig. 1).

Patients who consented to participate were assessed for eligibility criteria which included: age between 18 and 65 years old, diagnosis of schizophrenia according to DSM-IV-TR criteria confirmed by two senior psychiatrists, treated with clozapine for at least 18 weeks and being on a stable clozapine dose for at least four weeks prior to enrollment, serum 25(OH) vitamin D level below 75 nmol/l (30 ng/ml) and total severity of psychopathology score, as measured by the Positive and Negative Symptom Scale (PANSS) total score above 70. This threshold was chosen to ascertain that the patients had sufficient residual symptoms to be optimized by vitamin D addition and to avoid a floor effect. Exclusion criteria included: learning disability, organic brain disease, parathyroid disorder, inborn/acquired vitamin D metabolism disorders and patients already treated with vitamin D supplementation. The sample size was calculated as 45, based on an effect size of 0.6 ($\alpha = 0.05$, power $= 0.80$) of the primary outcome measure (reduction in total PANSS score).

The study was approved by GMHC's institutional review board and is registered on ClinicalTrials.gov (NCT01759485). All participants
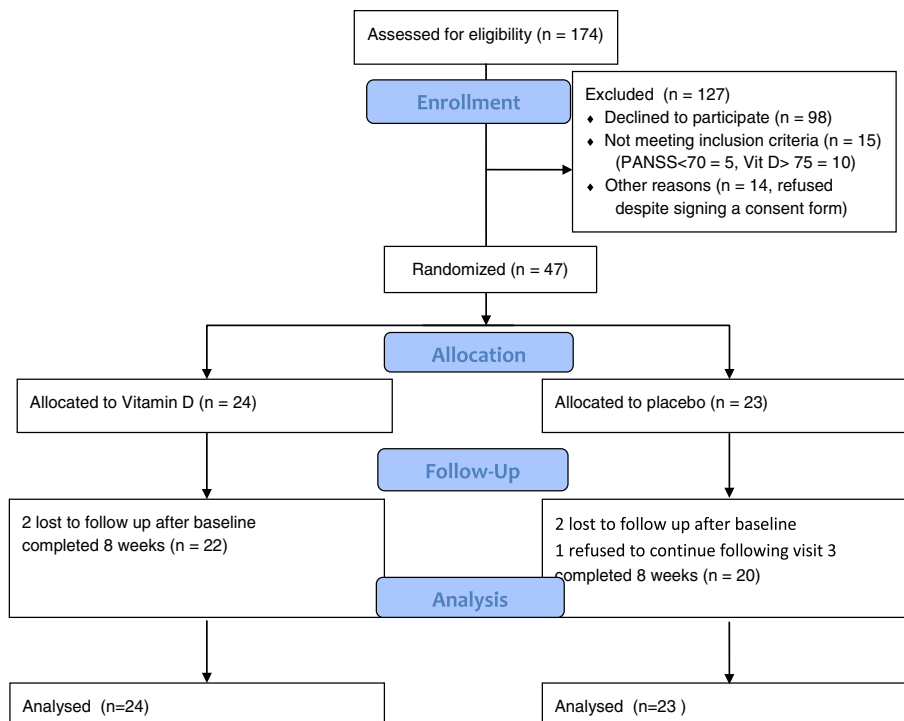

Fig. 1. CONSORT flow chart of the study recruitment and follow-up process.

provided a written informed consent after receiving an explanation on the nature of the study prior to partaking in it.

2.2. Investigational Medicinal Product and Masking

Both vitamin D and placebo were provided, in sealed, identical, opaque bottles, by Solgar Inc. (Petah-Tikva, Israel). The investigational medicinal products (IMP) were dispensed under the supervision of the study pharmacist. Upon recruitment, each patient was randomly assigned to either the vitamin D or the placebo group by a pre-prepared randomization code that was generated by the study pharmacist who was the only team member with an access to the information linking participants' details to their study codes. Randomization was based on 1:1 ratio between drug and placebo, and participants were sequentially assigned to the next treatment code of randomization. The IMP was administered on a weekly basis as oral drops (35 drops, 14,000 IU per week, 0.35 mg) by the study nurse in order to ensure adherence to medication. As there are no agreed guidelines as to which dose is relevant to the effects of vitamin D on the brain, the IMP dose was chosen according to previously reported guidelines to achieve sufficient increase in serum levels (van Groningen et al., 2010). A weekly-based administration was chosen to ascertain adherence to the study protocol.

2.3. Protocol and Design

The study was a parallel arm design, randomized, double-blind, placebo-controlled, prospective clinical trial.

Full assessments were carried out at baseline and after 8 weeks and included severity of the psychopathology, depression, cognition, metabolic parameters and safety measurements. Additionally, partial assessments that examined only severity of psychopathology and adverse events were done on a bi-weekly basis (visit 2 weeks, 4 weeks and 6 weeks).

2.4. Outcome Measures

The main outcome measure was the reduction in total Positive and Negative Symptoms Scale (PANSS) score. PANSS score ranges between 30 and 210 and patients were included in the study only if their baseline PANSS was above 70.

Secondary variables included: measures of severity of sub-scales of psychopathology – PANSS-positive (range 6–42), PANSS-negative (range 8–56) and PANSS-general psychopathology (range 16–102) sub-scores. Depressed mood was measured by the Calgary Depression Scale (CDS) which ranges between 0 and 27. Cognition was assessed using the Montreal Cognitive Assessment (MoCA) – a screening tool that has been proven to be sensitive in detecting minimal cognitive impairment in schizophrenia (Fisekovic et al., 2012). The instrument includes more items than the Mini-Mental Status Examination (MMSE) and also assesses executive functions relevant to schizophrenia such as abstraction and Clock Drawing Test. The questionnaire includes 10 items (2 visuospatial, 1 naming, 3 attention, 2 language, 1 delayed memory recall and 1 orientation) whose score ranges between 0 and 30. Metabolic assessment included body-mass index (BMI), waist circumference, blood pressure, pulse and blood tests for glucose, lipids and HbA1c.

Serum 25(OH) vitamin D levels were analyzed at the Biochemistry Laboratory, Rabin Medical Center, Belinson Campus, Petah Tikva, Israel using the ARCHITECT i2000SR immunoassay analyzer (Abbott, Abbott Park, Illinois, USA).

2.5. Safety

In each assessment, adverse events were screened using open questions and structured instruments such as the Sympson-Angus Scale (SAS) for extra-pyramidal side effects, Barnes Akathisia Rating Scale

(BARS) and Clozapine Adverse Events Inventory (CAEI). The CAEI was developed on the basis of the Glasgow Antipsychotic Side-effect Scale (GASS) (Waddell and Taylor, 2008) specifically for this study to monitor the existence and severity of 19 common clozapine side effects. Other measures of safety included an electrocardiogram to monitor QTc interval and routine clinical blood tests at baseline and at the eight week point.

2.6. Statistical Analysis

Data were analyzed using SPSS version 20 software (IBM Corporation, Armonk, New York). Univariate differences between groups were assessed using Student's t-test, Mann-Whitney test or χ^2 as appropriate. All patients who were randomly assigned to one of the two groups were included in the final analysis, as the study was designed as intention-to-treat with last observation carried forward in case of missing data. The superior effect of the drug over placebo on repeated measures over time was analyzed using General Linear Model with repeated measurements, taking into consideration time, effect of the drug and their interaction. Secondary outcome measures that were found to be significantly affected by vitamin D between the two time points (baseline and endpoint) were examined by post-hoc exploratory analysis, adjusting for age, sex, length of illness and season of study entry. Bonferroni post-hoc test for multiple comparisons was used as appropriate and significance levels were set accordingly ($p < 0.004$). We further performed a sub-analysis comparing supplementation with no supplementation in those with low baseline vitamin D levels (<50 nmol/l). In addition we assessed the possible correlation between change in vitamin D levels and change in the outcome measures for those with low baseline vitamin D.

3. Results

Out of the 174 screened patients, 47 (mean age 40.9 ± 1.5 years, range 22–65, 32 [68%] males, mean daily clozapine dose 414.4 ± 20, range 200–700 mg, mean duration of illness 16.2 ± 1.5 years) were randomly assigned to either the vitamin D group or the placebo group. See Fig. 1 for the specific reasons for the exclusion of 127 patients screened. Twenty four patients from the vitamin D group and 23 from the placebo group were analyzed using Intent-To-Treat design. The two groups' baseline characteristics are depicted in Table 1. There was no statistical difference in any of the baseline measures between the drug and placebo groups.

3.1. Vitamin D Levels

The mean serum 25(OH) vitamin D level of the whole sample ($N = 47$) at baseline was 39.8 ± 16.2 nmol/l (median 39.2, range 0–69.3). Baseline serum 25(OH) vitamin D levels of the two groups appear on Table 1. At baseline, 33 (70%) patients had insufficient vitamin D blood levels (i.e. <50 nmol/l). Of those, 10 patients (21%) were deficient (<25 nmol/l) with 3 (13%) patients in the placebo group and 7 (29%) in the drug group. The 25(OH) vitamin D serum levels increased significantly in the drug group (from 37.2 to 68.6, t(23) = −6.3, $p < 0.0001$) while essentially unchanged in the placebo group (from 42.7 to 42.3 nmol/l, t(22) = 0.114, $p = 0.91$). At endpoint, there were 4 (17%) patients with deficient levels of 25(OH) vitamin D in the placebo group and only one patient (4%) in the vitamin D group. This patient, despite attending all assessments and dosing visits, showed vitamin D level of 22 nmol/l at baseline and 17 nmol/l at endpoint due to unclear reasons.

3.2. Clinical Measures

No between group differences were found in the repeated measures model of PANSS total or in the sub-scores measures (Fig. 2). Using

Table 1
Baseline characteristics of the participants by study group.

	Vitamin D ($N = 24$)	Placebo ($N = 23$)	Statistics	p-Value
Age – mean	39.4 ± 9.6	42.5 ± 11.2	$t(45) = 1.02$	0.31
Age – range	26–63	22–65		
Sex – males (%)	18 (75)	14 (60.9)	$\chi^2(1) = 1.08$	0.36
Immigrants (%)	8 (33.3)	5 (21.7)	$\chi^2(1) = 0.37$	0.29
Singles (%)	19 (79.2)	16 (69.6)	$\chi^2(1) = 0.45$	0.34
Smoking (%)	13 (54.2)	11 (47.8)	$\chi^2(1) = 0.66$	0.44
Length of illness – (years)	15.3 ± 11.1	17.2 ± 9.5	$z(44) = -1.07$	0.29
Clozapine daily dose (mg)	414.6 ± 131.6	414.1 ± 131.6	$z(45) = -0.29$	0.77
Vitamin D (nmol/l)	37.2 ± 5.9	42.7 ± 15.5	$t(45) = 1.17$	0.25
PANSS total	81.8 ± 9.2	87.4 ± 13.7	$z(45) = -0.16$	0.11
PANSS positive	17.8 ± 5.1	19.9 ± 6.3	$z(45) = -1.12$	0.2
PANSS negative	26.6 ± 5.9	26.7 ± 4.6	$z(45) = -0.31$	0.76
PANSS general	37.1 ± 7.9	40.8 ± 8.1	$z(45) = -1.4$	0.16
Calgary Depression Scale	4.5 ± 4.2	5.2 ± 3.4	$z(45) = -1.22$	0.22
MoCA total score	21.7 ± 5.5	21.7 ± 4.7	$z(45) = -0.27$	0.8
BMI (kg/m^2)	28.3 ± 3.8	28.1 ± 6	$t(45) = -0.12$	0.9
Waist circumference (cm)	104.5 ± 9	99.1 ± 25.5	$t(45) = -0.44$	0.66
HDL (mg/dl)	49 ± 26.6	48.4 ± 22.4	$z(45) = -0.6$	0.95
Triglycerides (mg/dl)	176.8 ± 76.7	243.2 ± 229.5	$z(45) = -0.68$	0.5
Glucose (mg/dl)	111.8 ± 34	114.3 ± 60.3	$z(45) = -0.45$	0.65
HbA1C (%)	5.7 ± 0.7	6.0 ± 1.8	$z(45) = -0.48$	0.63
Systolic blood pressure (mmHg)	124.4 ± 9.8	128.3 ± 15.5	$t(45) = 1.02$	0.31
Pulse (bpm)	99.5 ± 18.3	99.7 ± 9.8	$t(45) = 0.06$	0.95

PANSS: Positive and Negative Symptom Scale; MoCA: Montreal Cognitive Assessment; BMI: Body Mass Index; HDL: High Density Lipoprotein.

general linear model with repeated measures, the only significant effect found was that of time, for both groups, but none of vitamin D X time interaction. PANSS total: $F(45) = 14.85$, $p < 0.0001$ for time, $F(45) = 0.17$, $p = 0.95$ for interaction, PANSS positive: $F(45) = 12.93$, $p < 0.0001$ for time, $F(45) = 0.67$, $p = 0.61$ for interaction, PANSS negative: $F(45) = 9$, $p < 0.0001$ for time, $F(45) = 0.5$, $p = 0.74$ for interaction and PANSS general psychopathology: $F(45) = 10.5$ $p < 0.0001$ for time, $F(45) = 0.25$, $p = 0.91$ for interaction. Furthermore, no significant association was found between the change in vitamin D serum levels from baseline to endpoint and PANSS total and sub-scores changes.

Comparison of the change in scores of the various outcome measures between baseline and endpoint (eight weeks) is presented in Table 2. There was a significant difference in the change of the MoCA total score between the vitamin D and placebo groups (0.9 vs -0.6, $p = 0.04$, respectively), however this effect is lost following Bonferroni correction for multiple comparisons. When tested using the general linear model with repeated measures, time was found to
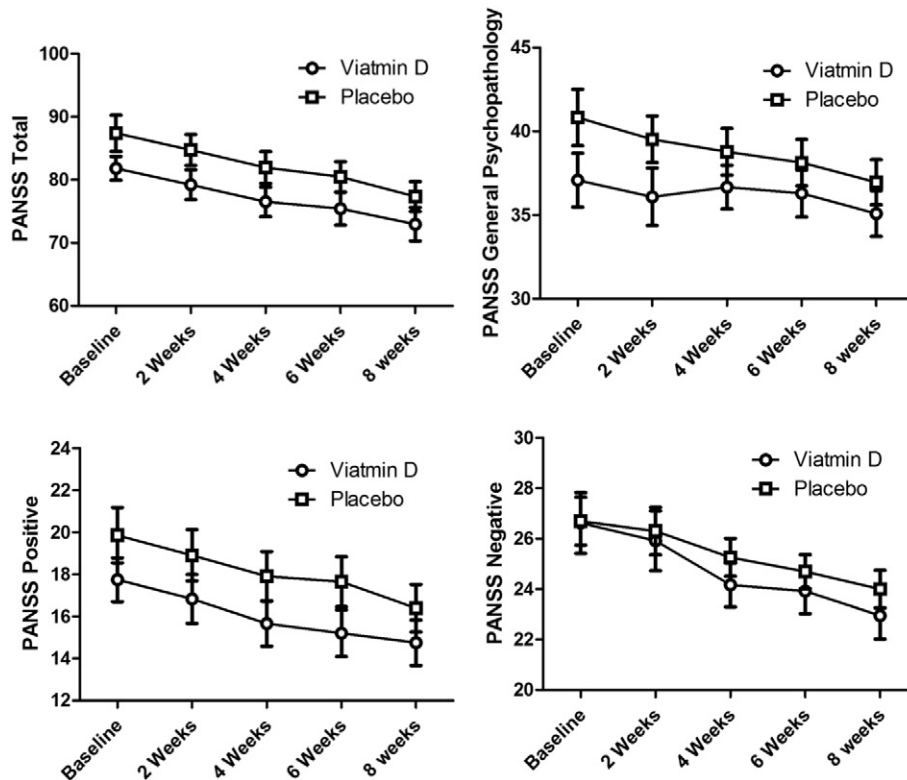

Fig. 2. Mean ($\pm$S.E) Positive and Negative Symptoms Scale (PANSS) total and sub-scores in the repeated assessments during the eight weeks of the trial, in the vitamin D ($n = 24$) and placebo groups ($n = 23$). There was no statistically significant difference between the placebo and vitamin D groups in any of the presented variables.

Table 2
Mean changes, between baseline and the eight week follow-up (two time points), in clinical and metabolic parameters of the vitamin D and placebo groups (endpoint-baseline scores).

	Vitamin D ($n = 23$)	Placebo ($n = 24$)	Statistics	p-Value	Effect size (Cohen's d)
PANSS total	−8.9 (7.3)	−10 (10.2)	$t(45) = 0.45$	0.65	−0.13
PANSS positive	−3 (2.1)	−3.5 (3.8)	$t(45) = 0.53$	0.59	−0.16
PANSS negative	−3.7 (4.1)	−2.7 (4)	$t(45) = -0.83$	0.41	0.24
PANSS general	−2 (8.4)	−3.9 (4.9)	$t(45) = 1.49$	0.14	−0.45
Calgary Depression Scale	−0.5 (2.7)	−0.8 (1.8)	$t(44) = 0.52$	0.6	−0.16
MoCA total score	0.9 (1.9)	−0.6 (2.9)	$t(44) = 2.1$	**0.04**[a]	**0.17**
BMI (kg/m^2)	−0.4 (1.6)	−0.1 (1.2)	$t(44) = 0.58$	0.56	0.18
Waist circumference (cm)	−0.4 (4.1)	−0.7 (7.9)	$t(44) = 0.17$	0.86	0.05
HDL (mg/dl)	−7 (27.3)	−3.1 (17.3)	$t(39) = -0.55$	0.58	0.18
Triglycerides (mg/dl)	11.5 (44)	−43.9 (130.8)	$t(39) = 1.83$	0.07	−0.63
Glucose (mg/dl)	4.3 (32.1)	−12.2 (44.7)	$t(44) = 1.44$	0.15	0.43
HbA1C (%)	−0.3 (0.4)	−1.1 (2.5)	$t(15) = 1.1$	0.35	−0.55
Systolic blood pressure (mmHg)	−1.6 (11.4)	−1.1 (16)	$t(44) = -0.13$	0.89	0.04
Pulse (bpm)	1.3 (15.4)	−3.5 (25.1)	$t(44) = 0.78$	0.43	−0.24

Bold: p < 0.05.
[a] Significance is lost following Bonferroni correction for multiple comparisons (significant value defined as $p = 0.004$).

be insignificant [F(44) = 0.21, $p = 0.65$], however, vitamin D × time interaction was significant [F(44) = 4.46, $p = 0.04$.]; though significance is lost following Bonferroni correction, since the adequate significance value was set to $p < 0.004$ (Table 2). Noteworthy, a multivariate regression analysis, adjusting for age, sex, length of illness and season at the study entry showed that vitamin D supplementation was associated with larger increase of MoCA score (B = 1.77, β = 0.18, SE = 0.7, $t = 2.52$, $p = 0.016$) from baseline.

Exploratory, post-hoc analysis was further performed to see the relative contribution of the MoCA components to the change in the composite score. Delayed recall (memory) and attention were found to have changed significantly in the vitamin D group but not in the placebo group, although significance was lost following Bonferroni correction for multiple comparisons of the MoCA components (the required $p = 0.007$; Fig. 3). Delayed recall item includes remembering five words during the interview. Point being given to every word remembered spontaneously without cue. Participants on vitamin D had an increased mean score on this item from 1.16 at baseline to 1.7 at endpoint ($z = -1.9$, $p = 0.049$) while participants on placebo had mean score of 1.55 at baseline and 1.45 at endpoint ($z = -0.36$, $p = 0.72$). Attention score is a summation of three items: forwards and backward digit span scored one point each, vigilance for sequence of letters scored one point and serial sevens scored up to three points. Participants on vitamin D increased significantly mean attention score from 4.17 at baseline to 4.58 at endpoint ($z = -2.23$, $p = 0.026$), while the increase in mean attention score in the participants on placebo (from 3.95 at
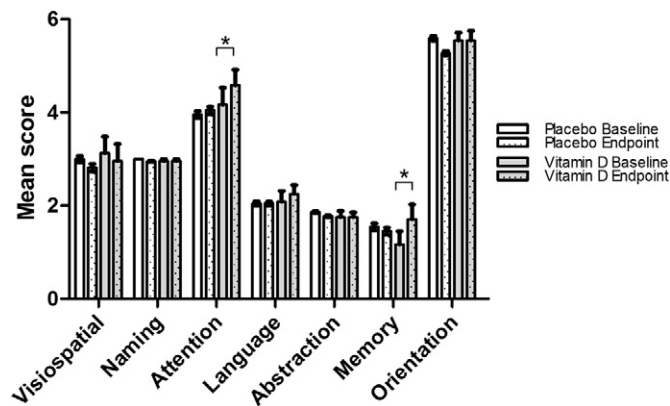
baseline to 4.04 at endpoint) did not reach statistical significance ($z = -0.35$, $p = 0.725$).

3.3. Metabolic Measures

There was no difference between the vitamin D and placebo groups with regard to change in metabolic parameters over time (see Table 2). Neither was there an effect of time on these parameters. There was also no linear association between the change in vitamin D serum levels and change in any of the metabolic parameters explored.

3.4. Adverse Events

Baseline rates of side effects were similar between groups. There were no severe adverse events noted during follow-up in both groups. As shown in Table 3, vitamin D group exhibited slightly more gastro-intestinal complaints: two patients reported nausea, three reported diarrhea and one reported abdominal discomfort. There was no significant difference between groups in the level of total CAEI score reduction (taking into account also the severity of adverse event): Vitamin D group 1.58 vs 1.77 placebo group, $t = 0.18$, $p = 0.86$).

Table 3
Adverse events reported by participants during the 8-week follow-up period. Numbers represents patients with reported item.

	Vitamin D ($n = 23$)	Placebo ($n = 24$)
Somnolence		
Slow thinking		
Restlessness		2
Confusion		
Palpitations		
Tachycardia		2
Shortness of breath	1	
Constipation		
Nausea	2	
Diarrhea	3	
Abdominal discomfort	1	
Salivation during day		
Salivation during night		1
Increased appetite	1	1
Weight gain		
Dizziness	2	
Tremor		1
Urine incontinence during day		
Urine incontinence during night		2
Total CAEI	−1.58	−1.78
SAS > 10	1	1
BARS > 0	1	

SAS: Simpson-Angus Scale; BARS: Barnes Akathisia Rating Scale; CAEI: Clozapine Adverse Effects Inventory.

Fig. 3. Mean (±S.E) scores of Montreal Cognitive Assessment (MoCA) domains in the vitamin D and placebo groups at baseline and endpoint (eight weeks). There was a significant increase in memory and attention scores in the vitamin D group only (*$p < 0.05$; however significance of these two domains is lost following Bonferroni correction for multiple comparisons: the required p value is $p = 0.007$).

3.5. Sub-Analysis According to the Change in Vitamin D Levels

One of the assumptions of the study was that the beneficial effects of Vitamin D will be more pronounced in those with low baseline vitamin D levels, who achieved normalization of vitamin blood levels following the supplementation. Therefore, we further performed sub-analyses for a subset of patients with pronounced low baseline vitamin D (<50 nmol/l, N = 33). We compared the psychiatric, cognitive and metabolic parameters between patients who were supplemented with vitamin D (n = 17) to those who were on placebo (n = 16). Sub-groups' characteristics appear on Supplementary Tables 1 and 2. There were no statistically significant differences between the sub-groups in changes of psychosis, mood and metabolic parameters, as well as MoCA scores, between baseline and endpoint. A significant increase in HDL levels was detected in patients who received vitamin D, but the significance is lost following Bonferroni correction for multiple comparisons (Supplementary Table 1; the required p is 0.003). Finally, we did not detect any significant correlation between changes in vitamin D levels and changes in outcome measures.

4. Discussion

This is the first study to explore the effect of vitamin D supplementation on schizophrenia patients in general and more specifically when used as an add-on to clozapine. After eight weeks of follow-up the main finding was that the effect of vitamin D on psychiatric symptoms and metabolic parameters did not differ significantly from that of placebo. It is possible that the robust decrease in the PANSS scores in both groups may have obscured an effect of vitamin D supplementation. A beneficial effect of vitamin D supplementation was noted on cognitive performance, as reflected in an increase in MoCA total score. However, this impact was with a small effect size (Cohen's d = 0.17) and was attributable to improvements in attention and memory domains only. Moreover, the significance of the improvements in the total MoCA and the two components was lost following Bonferroni correction for multiple comparisons.

The study sample demonstrated relatively high rate of low vitamin D levels. Of 62 patients assessed for eligibility to participate in the current study, 52 (83.8%) were found to have insufficient levels of vitamin D (<75 nmol/l). This high rate is within the range reported in a recent meta-analysis, that found an overall prevalence of vitamin D deficiency of 65.3% (95% CI 46.4%–84.2%) in schizophrenia patients (Valipour et al., 2014). Another study reported 86% in a cohort of patients with established psychosis (Lally et al., 2016), while another (Boerman et al., 2016) from the Netherlands, showed a rate of 34.9% deficient and 65.8% insufficient vitamin D levels among schizophrenia outpatients. Although Israel is sunny most of the year, the rate of vitamin D deficiency among schizophrenia patients was previously shown to be 57.1% (<40 nmol/l) (Itzhaky et al., 2012) and 98% insufficiency (<75 nmol/l).

Current evidence for vitamin D involvement in schizophrenia derives mainly from ecological data and association studies. A Finnish birth cohort study showed that vitamin D supplementation of at least 2000 IU per day to male infants during the first year of life reduced the risk of developing schizophrenia by 77% compared with those receiving lower daily doses (McGrath et al., 2004). Similarly, studies showed that low maternal vitamin D status may increase the subsequent risk of schizophrenia in the developing fetus (McGrath et al., 2010a). The authors were unable to find studies on the efficacy of vitamin D supplementation on psychosis. Notably, a recent study (Saad et al., 2016) showed a positive effect of vitamin D supplementation in young patients with autism, especially regarding behavioral measures. The current study did not demonstrate a differential effect of vitamin D in comparison to placebo regarding the impact on the severity of psychopathology in clozapine-treated patients. This lack of a beneficial effect in the current study may be attributed to the fact that the participants included treatment-resistant population that had been treated with several antipsychotic compounds for years (mean length of illness 16 years) and may thus be amenable to minor changes only. Positive effects of vitamin D may be more pronounced in early phases of schizophrenia, when the brain pathology is at its early stage.

In this study vitamin D was found mildly superior to placebo (effect size = 0.17) in improving cognition as measured by the total score of the screening tool MoCA, mainly with regard to attention and delayed recall domains. This finding should be considered with caution, as correction made for repeated measures led to loss of significance of the two cognitive components that were improved, and there is a risk of Type I error. However, taking into consideration the relatively small sample size, the short period of the trial and putatively the low dosage of vitamin D, this preliminary result may suggest that prolongation of the duration of exposure to vitamin D supplementation of a larger dose of vitamin D and a larger sample size might have shown a more robust effect. Consistent with our observation, epidemiological studies showed a positive association between vitamin D levels and cognitive performance, especially in older adults (Przybelski and Binkley, 2007; Wilkins et al., 2006). Recent data from a case-control study demonstrated that vitamin D levels correlated with negative and cognitive symptoms in first episode psychosis patients as opposed to a control group (Graham et al., 2015). Another cross-sectional study (Nerhus et al., 2017) showed that vitamin D deficiency in schizophrenia patients was significantly associated with decreased processing speed and decreased verbal fluency; however negative symptoms diluted that association. Moreover, in vitamin D deficient multiple sclerosis patients, three months of vitamin D supplementation was associated with a significant improvement in total MoCA and delayed recall scores (Darwish et al., 2017). However, a study in healthy subjects showed no superiority of vitamin D supplementation (5000 IU daily for six weeks) over placebo in improving cognitive performance (Dean et al., 2011). This may suggest that a beneficial effect of vitamin D on cognition is more apparent in cognitively impaired patients (such as subjects with schizophrenia) than in the normal functioning brain. As these results should be interpreted cautiously, future studies may be able to detect the effect more robust by using a more comprehensive cognitive assessment battery.

Heart failure, hypertension, stroke, and other cardiovascular diseases (CVD) in the general population have been associated with lower vitamin D levels (Liu et al., 2012) and there is an inverse relationship between serum vitamin D levels and hypertension, obesity and waist circumference (Lindqvist, 2014). A recent study, investigating a cohort of 324 chronic patients with psychosis (Lally et al., 2016) showed that 25(OH) vitamin D levels were negatively correlated with waist circumference, BMI, total cholesterol, triglycerides, HbA1c and hypertension. The current study however did not find any beneficial effect of vitamin D supplementation (and subsequent corresponding increase in serum 25(OH) vitamin D levels) on metabolic parameters. This may imply that the metabolic parameters are resistant to change in this unique population of chronic clozapine-treated patients. The lack of effect may also be related to the relatively short period of the study (eight weeks). Future supplementation studies should use larger doses of vitamin D and continue for longer follow-up periods.

5. Limitations

This is an innovative study, exploring for the first time, the effects of vitamin D in a population of treatment resistant schizophrenia patients maintained on clozapine. The results however, should be interpreted with caution due to the study's limitations. The study included a relatively small sample size, however, one should keep in mind that this is a population of severely ill patients, making recruitment of participants quite challenging. Moreover, most clozapine augmentation studies in the available literature had similar small sample sizes. Since patients with extreme presentation are less likely to consent to participate in a randomized controlled trial due to their paranoid and suspicious

attitudes, thus the study population may be slightly biased towards less severe cases. However, all patients had residual symptoms and they may indeed represent the substantial portion of chronic clozapine-treated patients who would eventually adhere to vitamin D supplementation.

Another limitation is the fact that no adjustment was done for ethnicity, which is an important factor in vitamin D skin synthesis. But, since this study included a quantitative measure of serum vitamin D that reflects both its synthesis and absorption, hence, ethnicity, as well as other factors that may affect absorption, seem to be less relevant.

6. Conclusions

This is the first study to explore the effects of vitamin D supplementation on schizophrenia patients in general and in clozapine-treated schizophrenia patients in particular. No significant advantage of vitamin D over placebo on psychotic, depressive and metabolic parameters was found. A mild positive effect was demonstrated on cognitive performance. Regression analysis, adjusting for age, sex, length of illness and season at the study entry showed that Vitamin D supplementation was a significant predictor of higher total MoCA score (B = 1.77, β = 0.18, SE = 0.7, t = 2.52, p = 0.016).Thus it appears that vitamin D supplementation may improve to some extent the cognitive performance in this population of clozapine-treated schizophrenia patients. However, further studies are needed to confirm such a pro-cognitive effect in earlier phases of the disorder, using a larger sample, a more sensitive cognitive assessment, longer duration and larger doses of vitamin D supplementation.

Conflict of interest

All authors declare no conflict of interest.

Authors' contribution

Amir Krivoy - Study design, PI, data collection, analysis, manuscript drafting.

Roy Onn - Study design, co-PI, literature search, data collection, manuscript drafting.

Yael Vilner - Data collection, Data management, Data analysis, manuscript drafting.

Eldar Hochman - Study design, Data collection.

Shira Weizman, Amir Paz, Shmuel Hess, Roi Sagy, Shiri Kimhi-Nesher, Ehud Kalter, Tal Friedman, Sharon Trommer - Data collection, Data management.

Gil Bormant, Zvi Friedman - Study coordination, Data collection, Data interpretation.

Avi Valevski, Abraham Weizman - Study design, Supervision, Data interpretation, Critical review of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2017.11.027.

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