

Low Serum Vitamin D Concentrations in Patients with Schizophrenia

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ABSTRACT: **Background:** Vitamin D is increasingly associated with the pathology of cognition and mental illness. Vitamin D receptors have been detected on neurons that regulate behavior.

Objective: To assess vitamin D serum concentrations in patients with major depression and schizophrenia as compared to healthy controls and to determine if a correlation exists between serum levels of vitamin D and disease activity.

Methods: We recruited 50 patients with schizophrenia and compared them to 33 patients with major depression and 50 controls with no major psychopathology. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia and the Hamilton Depression scale for depression were administered on the same day the blood samples were drawn. We used LIAISON® 25-OH vitamin D (DiaSorin) immunoassay to measure serum concentrations of 25-OH vitamin D.

Results: Lower serum vitamin D concentrations were detected among patients with schizophrenia (15.0 ± 7.3 ng/ml) compared to patients with depression (19.6 ± 8.3 ng/ml) and to controls (20.2 ± 7.8 ng/ml, $P < 0.05$). We found no correlation between disease activity, measured by the PANSS score, and vitamin D levels.

Conclusions: Serum vitamin D levels were lower in patients with schizophrenia as compared to patients with depression and to healthy controls. No correlation was found between serum concentration and disease activity. Additional studies are needed to elucidate the role of vitamin D in the autoimmune mechanism and in the pathogenesis of schizophrenia.

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KEY WORDS: vitamin D, autoimmune diseases, schizophrenia, depression

Vitamin D is involved in many metabolic functions in addition to bone and calcium metabolism; diverse cells carry vitamin D receptors on their membranes [1,2]. There is mounting evidence that vitamin D supplementation reduces overall mortality [3]. Other reports (mainly retrospective) indicate that

adequate vitamin D serum levels are associated with protection from various malignancies, thrombosis and cardiovascular diseases [2,4,5]. On the other hand, vitamin D deficiency was found to be related to autoimmunity and immune cell overactivity and was observed in autoimmune animal models of diabetes, rheumatoid arthritis and other autoimmune disorders [1,2,6,7].

Lower levels of vitamin D were significantly more prevalent among patients with multiple sclerosis and preceded the development of the disease by some years [8]. Several authors investigated the link between vitamin D and the central nervous system. Vitamin D is increasingly associated with impaired cognitive abilities, and vitamin D receptors have been detected on neurons that take part in behavior control and regulation. Vitamin D supplementation during early fetal growth and early childhood was shown to have a positive effect on brain development and later mental functioning [2,9].

Autoimmune mechanisms have also been implicated in the pathogenesis of schizophrenia. Multiple studies have elaborated on the various immunological aspects detected among patients with schizophrenia: namely, antibodies to neurotransmitter receptors and cytokines, the appearance of antinuclear antibodies in patients with schizophrenia, along with increased concentration of interleukin-3-producing CD5+ B cells [10-14].

Data regarding vitamin D levels specific to the Israeli population of psychiatric patients are lacking. The aim of the present study, therefore, was to assess vitamin D levels in patients with major depression and schizophrenia compared to healthy controls. We also aimed to investigate possible correlations between serum concentrations of vitamin D and disease activity. We also measured standard autoimmune parameters such as anticardiolipin antibodies (immunoglobulin G and M) in order to detect their possible involvement in the emergence of schizophrenia.

PATIENTS AND METHODS

PATIENTS' SERA

Sera were collected at the Ness Ziona Mental Health Center, Israel. We recruited 50 patients with schizophrenia, 33 patients

*The first two authors contributed equally to this study

with major depression, and 50 controls (health center personnel) with no major psychopathology. All subjects were age-matched adults who consented to participate in the study. The study received approval by the local ethics committee.

CLINICAL AND PSYCHIATRIC EVALUATIONS

- Demographic details – age, gender, ethnicity, occupation, living arrangements, marital status, and body mass index
- Subjective evaluation of daily duration of exposure to sun
- Clinical manifestations of disease – diagnosis, age at first psychiatric diagnosis, number of hospitalizations, duration, and type of psychiatric and non-psychiatric medications used, clinical manifestations, and family mental health history.

To rule out the possible effect of medications as a cause of the difference in vitamin D levels between the three groups, we categorized the patients according to medications taken. The schizophrenic patients were divided into five subgroups according to the different combinations of medications: antipsychotic, mood stabilizers, etc. Only one of the 50 patients was not taking any medication.

- Nutritional assessment (fish and oils, meat, eggs) – a dietary assessment completed by each patient regarding his or her intake of food containing vitamin D
- Clinical assessment – the Clinical Global Impression scale was used to evaluate the level of general functioning. The CGI is a standardized assessment tool that allows the clinician to rate illness severity. This scale consists of three global subscales formatted for use with the Global Scoring Sheet. The scoring values are on a Likert scale ranging from 1 to 7.

PANSS AND HAMILTON SCALES

The Positive and Negative Syndrome Scale for schizophrenia and the Hamilton Depression Scale were administered the same day the blood sample was drawn. These are validated scales and are customarily used to quantify the intensity of psychotic and depressive conditions respectively. Briefly, the PANSS is used for measuring symptom severity in patients with schizophrenia and evaluating positive and negative symptoms of psychotic disorders [15]. The Hamilton Depression Scale is used to rate the severity of depression in patients who have been diagnosed as depressed. The higher the score, the more severe the depression [16].

VITAMIN D MEASUREMENT

We used LIAISON® 25-OH vitamin D Immunoassay (DiaSorin, Stillwater, MN, USA) to measure serum concentration of 25-OH vitamin D. Briefly, this method for quantitative determination of 25-OH vitamin D is a direct, competitive chemiluminescent immunoassay. A specific antibody to

vitamin D is used to coat magnetic particles (solid phase) and vitamin D is linked to an isoluminol derivative. During incubation, 25-OH vitamin D is dissociated from its binding protein and competes with labeled vitamin D for binding sites on the antibody. After incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units and is inversely proportional to the concentration of 25-OH vitamin D present in calibrators, controls, or samples.

ANTICARDIOLIPIN IgM AND IgG ANTIBODY MEASUREMENT

We used the LIAISON® for the quantitative determination of the autoantibody titers. This is a two-step immunoluminometric sandwich assay that employs directly coated magnetic microparticles.

STATISTICAL ANALYSIS

Prevalence rates between the groups were compared using the chi-square and Fisher exact tests (two-tailed), as appropriate. Continuous variables are expressed as mean ± standard deviation and were compared between the three groups by one-way ANOVA with multiple comparisons by Bonferroni. Student’s *t*-test was used to compare continuous variables in two groups. Pearson correlations were performed for associations between variables. We used multiple linear regression to look for predictive variables for anticardiolipin antibody level. For all tests, a *P* value < 0.05 was considered statistically significant. SPSS-17 was used for data analysis.

RESULTS

PATIENTS WITH SCHIZOPHRENIA

Overall, 50 patients with schizophrenia were enrolled. Their average age was 40.2 ± 13.4 years (range 19–65 years). There were 34 males (68%) and 16 females (32%). The mean age of disease onset was 24.8 ± 10.5 years and disease duration was an average of 15.4 ± 12.6 years. The average PANSS score was 22.5 ± 88.5; positive psychotic symptoms scored 19.6 ± 8.1 and negative symptoms 28.6 ± 7.7.

PATIENTS WITH MAJOR DEPRESSION

For comparison, we enrolled 33 patients with major depression. One patient was eliminated from the study because of technical problems with the laboratory testing. Their average age was 48.5 ± 11.4 years (range 18–65 years), with 11 males (33%) and 22 females (67%). The mean age at disease onset was 48.4 ± 11.8 years and disease duration was 10.2 ± 10.4 years. Their average Hamilton score was 19.1 ± 11.0: 34 males (68%)

CGI = Clinical Global Impression scale

Ig = immunoglobulin
PANSS = Positive and Negative Syndrome Scale

and 16 females (32%). Their mean age at disease onset was 24.8 ± 10.5 years and disease duration was 15.4 ± 12.6 years.

As expected, the CGI of healthy controls was 1.0, whereas patients with schizophrenia had a more severe disease, reflected by a CGI score of 5.6 ± 0.9 , compared to 3.6 ± 1.4 in patients with depression ($P < 0.01$).

HEALTHY CONTROLS

For a second comparison we recruited 50 healthy controls, 13 males (26%) and 37 females (74%), and their age was 39.7 ± 10.7 years.

DAILY DURATION OF SUN EXPOSURE

There were no significant differences in the average patient estimates of daily sun exposure; patients with schizophrenia estimated that the time spent exposed to sun was 1.7 ± 2.2 hours, compared to 1.0 ± 1.0 hours in patients with depression and 1.2 ± 1.2 hours in healthy controls [Table 1].

Table 1. Average daily sun exposure (in hours) estimated by study participants

	No. of subjects	Mean \pm SD (hrs)	P value
Controls	50	1.2 ± 1.2	Not significant
Depression	32	1.0	
Schizophrenia	50	1.7 ± 2.2	

Figure 1. Vitamin D concentration in the three study groups

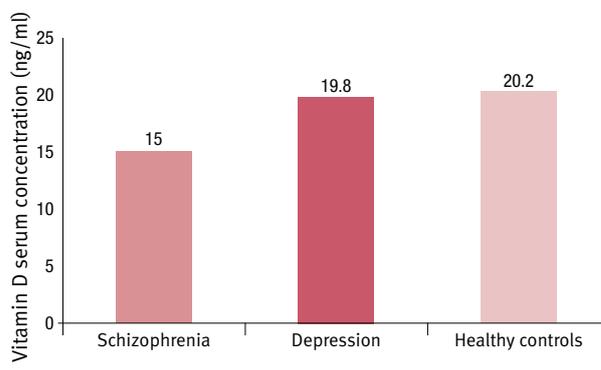


Table 2. Vitamin D status according to levels in the study population*

Vitamin D status	Schizophrenia	Depression	Controls	Total
Deficient (< 15 ng/ml)	28 (57.1)	11 (33.3)	12 (24.5)	51 (39.0)
Insufficient (15–30 ng/ml)	20 (40.8)	18 (54.5)	31 (63.3)	69 (53.0)
Sufficient (> 30 ng/ml)	1 (2.0)	4 (12.0)	6 (12.2)	11 (8.0)

The values presented are the number (and percentage) of subjects

* $P < 0.05$

EFFECT OF NUTRITION ON VITAMIN D CONCENTRATIONS

We could not detect any significant impact of diet composition regarding vitamin D-containing elements on the concentration of serum vitamin D. We did, however, observe a mild difference between the percentage of egg consumption (92% of schizophrenic patients compared to 76% of depression patients), but their correlation to vitamin D serum concentration was no more than borderline ($P = 0.08$). Within the schizophrenic group, we found higher vitamin D concentrations among fish eaters, 17.3 ± 8.2 ng/ml (25 patients) compared with non-fish eaters, 11.7 ± 5.4 ng/ml (24 patients) ($P = 0.02$).

According to the nutrition survey, four patients in the depression group and one of the healthy controls consumed vitamin D supplements.

VITAMIN D SERUM CONCENTRATIONS

Patients with schizophrenia had significantly lower serum vitamin D levels compared to patients with depression and to controls (15.0 ± 7.3 , 19.6 ± 8.3 , 20.2 ± 7.8 ng/ml respectively, $P < 0.05$). There was no significant difference between vitamin D levels in the depression group compared to healthy controls [Figure 1].

When patients were grouped according to vitamin D ranges (deficient < 15 ng/ml, insufficient 15–30 ng/ml, sufficient > 30 ng/ml), the schizophrenia group comprised a greater proportion of all vitamin D-deficient patients compared to vitamin D-insufficient patients – 28/49 (57.1%) vs. 20/49 (40.8%) respectively [Table 2].

COMPARISON OF VITAMIN D LEVELS ACCORDING TO TYPE OF THERAPY

To analyze the possible effects of concomitant medications on serum vitamin D concentrations, we defined four main categories of drug types: antipsychotics, antidepressants, sedatives, mood stabilizers. We did not find any relationship between the medication type or their combinations and vitamin D serum concentration (data not shown).

VITAMIN D LEVELS AND DISEASE ACTIVITY

The level of vitamin D did not correlate with disease duration, CGI scores or PANSS scores.

CARDIOVASCULAR MORBIDITY

Analysis of other non-psychiatric clinical manifestations in all subjects showed a greater proportion of cardiovascular disease in the patient groups compared to controls. Since an association of cardiovascular disease with low vitamin D levels has been reported, we investigated this in our study population. All subjects were categorized according to cardiovascular medical history. Among the patients with depression, significantly lower vitamin D concentrations were recorded among those with existing cardiovascular disease [Table 3].

Table 3. Association between concomitant cardiovascular disease and vitamin D serum concentration

	Cardiovascular manifestation	No. of subjects*	Vitamin D (ng/ml)	P value
Controls	No	45	19.9 ± 7.8	0.434
	Yes	4	23.1 ± 7.8	
Depression	No	20	21.6 ± 8.9	0.043
	Yes	8	14.4 ± 4.9	
Schizophrenia	No	39	14.6 ± 7.5	0.408
	Yes	9	16.9 ± 7.0	

ANTICARDIOLIPIN ANTIBODY LEVELS

We did not detect any differences between the three study groups regarding levels of anticardiolipin IgG or IgM. Levels of IgG aCl in the schizophrenia group did not differ from those recorded in patients with depression or with the healthy controls: 4.1 ± 8.3, 2.6 ± 1.8, and 3.5 ± 5.2 GPL (IgG phospholipid) U/ml, respectively. Similarly, IgM aCl levels in the schizophrenia patients did not differ from those measured in patients with depression or from the healthy controls: 3.8 ± 7.6, 2.4 ± 1.0 and 2.9 ± 2.1 MPL (IgM phospholipid) U/ml, respectively.

In the schizophrenia group, disease activity (PANSS) significantly correlated with titers of cardiolipin IgM antibody level (*P* = 0.012).

DISCUSSION

The etiology of schizophrenia is multifactorial. Acquired, genetic and environmental factors have been implicated in its pathogenesis, as were infections and autoimmune reactivity [17]. Vitamin D is believed to have therapeutic benefits. It has been reported that vitamin D activates receptors on neurons located in regions that are implicated in behavior regulation; it stimulates neurotrophin release and protects the brain by enhancing antioxidant and anti-inflammatory defenses against vascular injury [18].

A study conducted in Denmark demonstrated that neonates with low vitamin D concentrations had a significantly increased risk of schizophrenia (up to twofold) compared to neonates with concentrations ranging between 40.5 and 50.9 nmol/L. Interestingly, neonates with excessive concentrations of vitamin D also had an elevated risk for this disorder [19]. Additional epidemiological data demonstrate an increased prevalence of schizophrenia that developed among neonates born in winter [20], which may be attributed to low vitamin D levels occurring more often during that season.

Our results indicate that adults with schizophrenia also have significantly lower serum concentrations of vitamin D compared to healthy controls and to patients with major depression. This finding remained after evaluating the impact

of various factors such as self-reported sun exposure, consumption of various medications and food supplements, and analysis of diet composition.

Few researchers have dealt with the putative association between serum vitamin D concentration and mental illnesses. In two studies that primarily aimed to explore the link between vitamin D and osteoporosis in patients with schizophrenia, low vitamin D concentrations were recorded [21,22].

One would expect that vitamin D levels in schizophrenic patients in Israel would be higher due to the sunny climate. However, patients in our study were recruited from a closed psychiatric ward where mobility is restricted. It is likely that most of these patients estimated their daily sun exposure at the level achieved prior to their hospitalization, when they were recruited for this study. Interestingly, Oren et al. [23] presented a study aimed at assessing the status of vitamin D in Israel. They reported that although Israel is situated in a coastal area and is characterized mostly by sunny weather year round, vitamin D deficiency is prevalent. In their study, 78% of the 195 subjects who participated had insufficient vitamin D serum concentrations (< 75 nmol/L), and 27% of them were also vitamin D deficient (< 37.5 nmol/L). They found that in Israel, vitamin D insufficiency is widespread across all ages and ethnic groups, genders, and seasons of the year.

The fact that patients with depression had vitamin D levels equal to that of the controls is probably related to the fact that this cohort was more heterogeneous than the patients with schizophrenia. Some patients were recruited from the outpatient clinic, reflecting a milder disease. Therefore, it seems reasonable that their daily habits and activities were more similar to those of the healthy controls, leading to comparable vitamin D levels.

As mentioned above, low levels of vitamin D were found to be a risk factor for different diseases, including those involving the central nervous system. A study to investigate bone metabolism in multiple sclerosis patients revealed that 77% of the patients had insufficient serum 25-OH vitamin D levels (< 50 nmol/L) [24]. Munger and collaborators [8] reported that among white, active-duty, U.S. military personnel, the risk of developing multiple sclerosis significantly decreased with increasing levels of 25-OH vitamin D.

Despite the low vitamin D concentrations we observed in patients with schizophrenia, we failed to demonstrate a correlation between vitamin D levels and disease activity measured by the PANSS score, which estimates the severity of this psychotic condition. This score is well-validated for schizophrenia [15]; however, it does not seem to touch on the relevant aspects that associate this condition with vitamin D concentration.

We also found a high prevalence of cardiovascular diseases in our patient groups compared with the controls. A high risk for cardiovascular disease was reported in schizophrenia [25]. We recorded an inverse association between vitamin D

aCl = anticardiolipin

concentrations and comorbid cardiovascular conditions in the depression subgroup only [Table 3]. This may be related to the patients' higher average age and the higher rates of cardiovascular morbidity in this study.

As mentioned above, autoimmune mechanisms may be involved in the pathogenesis of schizophrenia [10]. We did not find elevated titers of anticardiolipin antibody in our patient groups. Our findings are compatible with previous publications, yet the literature varies regarding this issue [12].

Our study detected a positive correlation (although within normal levels) between anticardiolipin antibody titers and severity of schizophrenia. The significance of this finding needs to be further investigated.

CONCLUSIONS

Low serum vitamin D levels may play a role in the pathogenesis of schizophrenia. Based on our findings, we advocate routine testing of vitamin D concentrations in these patients and supplementation as needed.

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Capsule

Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles

Innate lymphoid cells (ILC) expressing the transcription factor ROR γ t induce the postnatal formation of intestinal lymphoid follicles and regulate intestinal homeostasis. ROR γ t+ ILC express the aryl hydrocarbon receptor (AhR), a highly conserved, ligand-inducible transcription factor believed to control adaptation of multicellular organisms to environmental challenges. Kiss et al. show that AhR is required for the postnatal expansion of intestinal ROR γ t+ ILC and the formation of intestinal lymphoid follicles. AhR activity within ROR γ t+ ILC

could be induced by dietary ligands such as those contained in vegetables of the family Brassicaceae. AhR-deficient mice were highly susceptible to infection with *Citrobacter rodentium*, a mouse model for attaching and effacing infections. These results establish a molecular link between nutrients and the formation of immune system components required to maintain intestinal homeostasis and resistance to infections.

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Eitan Israeli