Comprehensive Psychoneuroendocrinology 10 (2022) 100126

Contents lists available at ScienceDirect

Comprehensive Psychoneuroendocrinology

journal homepage: www.sciencedirect.com/journal/comprehensive-psychoneuroendocrinology

25(OH)D levels are decreased in patients with difficult-to-treat depression

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ARTICLE INFO

Keywords:
MDD
Vitamin D
25(OH)D
Suicidal ideation
Depressive symptoms

ABSTRACT

Objectives: The aims of the study are i) to compare 25-hydroxyvitamin D (25(OH)D) levels between clinically depressed individuals with insufficient treatment response and healthy controls and ii) to test the association between 25(OH)D levels and different affective disorder diagnoses (i.e., major depressive disorder (MDD) single episode, MDD recurrent episode, chronic MDD, and dysthymia), as well as grade of suicidal ideation.
Method: We quantified serum 25(OH)D in 202 individuals with difficult-to-treat depression (DTD) and 41 healthy controls. Patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV-TR). ANCOVA was used to test differences in mean 25(OH)levels between depressed and controls, adjusting for sex, age, smoking, sampling season, ethnicity, somatic illness, and body mass index (BMI). Binary logistic regression models were used to test the association between depression and 25(OH)D levels.
Results: Patients with difficult-to-treat depression had significantly lower levels of 25(OH)D compared to healthy controls (ANCOVA, $F = 4.89$; $p = 0.03$). Thirty percent of the depressed patients were 25(OH)D deficient (<50 nmol/L) compared to 5% of the controls (Chi-squared test, $\chi^2 = 11.38$; $p < 0.01$). The odds for being depressed decreased significantly with 17% per 10 nmol/L increase of 25(OH)D (Binary logistic regression, $p < 0.05$).
Limitations: The cross-sectional design of the study precludes any conclusions about causality. A large part of the patients took psychotropic drugs and/or had somatic illnesses, which might have affected the results.
Conclusion: The results of the present study add to the body of evidence linking 25(OH)D deficiency and depression. Further investigations are warranted to better understand any clinical implications of this association.

1. Introduction

A growing body of evidence indicates that 25-hydroxyvitamin D (25(OH)D) deficiency may be involved in the pathogenesis of depression. Consequently, assessment of 25(OH)D status, as well as the potential utility of 25(OH)D supplements, has become of increasing interest among psychiatrist and other medical professions [1–3]. Many cross-sectional studies have reported lower levels of 25(OH)D in depressed patients compared to healthy controls [4] but there are some studies showing opposite results [5–7]. Possible reasons behind these discrepancies across studies may involve methodological differences such as variations in sample sizes, inclusion criteria, definitions of 'depression', definitions of 25(OH)D deficiency, and other study-specific confounding factors. It is possible that low levels of 25(OH)D contribute to the psychopathology only in certain subgroups of depressed patients and only at certain levels of deficiency [3]. Some studies suggest that lower 25(OH)D levels are associated with more severe depressive profiles [4,8] or specific types av depressions, such as suicidal depression [9,10] or "inflammatory depression" [4,9,11,12]. In order to move towards "personalized psychiatry", it is important to test associations between biomarkers, in this case 25(OH)D, and different clinical phenotypes of depression. There is also a need to investigate the association between 25(OH)D and depression in large-scale and clinically well-characterized cohorts.

The aim of this study was to assess the relationship between serum 25(OH)D and depression in a large cohort of clinically depressed individuals with insufficient treatment response. Our intention was to study 25(OH)D in different affective disorder diagnoses groups, as well as 25(OH)D's relation to different aspects of suicidality. We hypothesized that there would be a difference in 25(OH)D levels between

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https://doi.org/10.1016/j.cpnec.2022.100126
Received 27 December 2021; Received in revised form 7 February 2022; Accepted 7 February 2022
Available online 9 February 2022

depressed patients and healthy controls, but that the results would vary dependent on type of depression. We also hypothesized that low 25(OH)D levels would be associated with symptom severity and/or grade of suicidal ideation.

2. Materials and methods

This study was approved by the Regional Ethical Review Board in Lund, Sweden (# 2011/673; amendment # 2012/523). Before inclusion, oral and written information describing the purpose of the study was provided, and each participant gave their written informed consent.

2.1. Participants

The current study sample consisted of 202 patients and 41 healthy controls. Demographic characteristics are described in Table 1. The current study sample is a subset of the Genes, Depression, and Suicidality (GEN-DS) study. A detailed presentation of the study protocol has been described elsewhere [13].

Table 1
Patient characteristics and group differences for MDD subjects and healthy controls.

Variables	MDD (n = 202)	Healthy Controls (n = 41)	p-value
Sex (% women)	62%	73%	0.22
Age years (median (min-max))	38.2 (18–77)	35.2 (21–66)	0.22
BMI, mean (kg/m^2), (SD)	25.9 (4.9)	23.5 (4.2)	<0.01**
Smoker (% yes)	21%	8%	0.05*
Ethnicity (%) [a]			
Caucasian	94%	95%	0.70
Other	6%	5%	
Medication			
Psychotropic drugs	81%	0%	
Anticonvulsants	25%	0%	
Sample season (% summer)	47%	63%	0.07
Somatic illness (% yes)	64%	0%	
25(OH)D, mean (nmol/L), (SD)	63.7 (24.1)	72.9 (18.0)	0.02*
25(OH)D deficiency (% <50 nmol/L) [b]	30%	5%	<0.01**
Somatic illness			
Infectious diseases (n = 1)	1%	N/A	
Tumors (n = 2)	1%	N/A	
Blood and blood-forming organs (n = 6)	3%	N/A	
Endocrine, nutrition and metabolic disorders (n = 22)	11%	N/A	
Nervous system disorders (n = 27)	14%	N/A	
Ophthalmologic disorders (n = 2)	2%	N/A	
Ear related diseases (n = 5)	3%	N/A	
Circulatory organ disorders (n = 18)	9%	N/A	
Respiratory system disorders (n = 16)	8%	N/A	
Digestive system disorders (n = 34)	17%	N/A	
Skin diseases (n = 20)	10%	N/A	
Muscular-skeletal system disorders (n = 47)	23%	N/A	
Urine and sexual organ disorders (n = 9)	5%	N/A	
Congenital anomaly and chromosomal aberration (n = 0)	0%	N/A	
Current injury or intoxications (n = 0)	0%	N/A	
Other, unspecified somatic conditions (n = 11)	6%	N/A	

* Significant at the 0.05 level (2-tailed).
** Significant at the 0.01 level (2-tailed).
Acronyms: major depressive disorder (MDD), body mass index (BMI), standard deviation (SD), non-applicable (N/A).
[a] 'Other' includes Latin-American and Asian origin, as well as missing data (n = 13).
[b] 50 nmol/L = 20 ng/ml.

Patients were referred to the study from four special psychiatric care clinics in southern Sweden. A semi-structured research protocol was used to collect data on previous and current psychosocial circumstances, previous and current psychiatric treatments, on-going and previous psychiatric symptoms as well as history of suicide attempt and present suicidal ideation, on-going and previous alcohol and drug use, and on-going somatic diagnoses and treatments. Patients previously diagnosed with an affective disorder, with an insufficient treatment response, were included in the GEN-DS study. Insufficient treatment response was defined as not having achieved remission with previous and ongoing treatments during the current depressive episode [13]. Remission was defined according to Ref. [14]; i.e., ascribed after 3 consecutive weeks during which minimal symptom status is maintained (absence of both sadness and reduced interest/pleasure, along with the presence of fewer than three of the remaining seven DSM-IV-TR diagnostic criterion symptoms) [14]. Patients in the present study were diagnosed with major depressive disorder (MDD) single episode (n = 17), MDD recurrent episode (n = 101), chronic MDD (n = 59) or dysthymia (n = 18). In cases where the patients had more than one axis I diagnose according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), only the main axis I diagnosis was considered in the analyses. In DSM-V, chronic MDD and dysthymia are consolidated into the same diagnose, namely 'Persistent Depressive Disorder (Dysthymia)' [15]. Since we used DMS-IV-TR diagnoses as clinical evaluation in the study, depressed mood for longer than two years was classified as chronic depression or dysthymia dependent on if the episode started as a clinical depression or not [16].

Chronic depression was defined as having a depression with more severe symptoms than in dysthymia, for two years or more, without at least two months in remission during the depressed period. Thus, most patients had a chronic illness course, to various degrees, and the sample was in accordance with the 'real-life' heterogenous population generally seeking psychiatric care. Patients were enrolled between 2012 and 2020 and exclusion criteria from the current study were a body mass index (BMI) of less than 15, pregnancy, current liver disease or bipolar type I or II diagnosis. Psychotic features were not an exclusion factor, and none of the patients, but one, had any distinct depressive delusions according to the Comprehensive Psychopathological Rating Scale (CPRS) [17]. In addition, taking 25(OH)D supplements was added as exclusionary criteria in the present study. Eighty-one percent of the patients were treated with psychotropic drugs, and 25% were treated with anticonvulsants drugs (carbamazepine, clonazepam, lamotrigine, topiramate or valproic acid), either as additional treatment to other psychotropic drugs (90%) or as single treatment (10%). Anticonvulsants might affect 25(OH)D levels and are sometimes added to antidepressant treatment to achieve better treatment effects [18]. Therefore, we compared 25(OH)D levels between those who were treated with anticonvulsants and those who were not.

Healthy controls were recruited through advertisements in newspapers, social media, and flyers. All controls underwent a clinical psychiatric and somatic evaluation including a standardized diagnostic assessment based on the Mini-International Neuropsychiatric Interview (MINI). Exclusion criteria were previous or present psychiatric illness, previous or present addiction disorder, previous or present treatment with psychotropic drugs or psychotherapy, severe or instable somatic illness, ongoing infection, present pregnancy or breast-feeding and treatment with certain blood- or immuno-related drugs. The healthy controls were given a small monetary compensation after the blood draw.

2.2. Measures

2.2.1. Diagnostic assessment

After inclusion in the study, patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV-TR). The diagnostic procedure for all patients included the Mini

International Neuropsychiatric Interview (MINI) 6.0 [16] and the Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II) [19].

2.2.2. 25(OH)D assays; 25(OH)D_2 and 25(OH)D_3

Serum was collected in serum separator tubes, protected from light by aluminum foil, and stored at −80 °Celsius until assay. The time of storage (6 months−7 years) is not likely to have had an impact on the result of the analysis due to the relatively stable 25(OH)D molecule [20] Analyses of 25(OH)D_2 and 25(OH)D_3 were done by liquid-chromatography-mass-spectrometry, model Sciex API 4000 LC/MS/MS (MA, USA). Coefficient of variation (CV) values were as follows: for 25(OH)D_2; 6,0% at 35 nmol/L and 5% at 114 nmol/L, and for 25(OH)D_3; 8% at 33 nmol/L and 5% at 133 nmol/L. The lowest detection limit is 6 nmol/L for both for 25(OH)D_2 and 25(OH)D_3. The analyzes were conducted by the department of clinical chemistry at Scania University Hospital, which is accredited by SWEDAC (the Swedish Board for Accreditation and Conformity Assessment). In line with clinical guidelines [21], in cases of a 25(OH)D_2 level >10 nmol/L, the 25(OH)D_2 levels were added to the 25(OH)D_3 levels in the statistical analysis, i.e., while describing 25(OH)D in the article, the sum of 25(OH)D_2 and 25(OH)D_3 is referred to as 25(OH)D. Twenty-four MDD subjects had 25(OH)D_2 levels >10 nmol/L (median 25(OH)D_2 level: 16.2 nmol/L). 25(OH)D data displayed a normal distribution, and all samples were above detection limit. Blood sampling was performed across the year. Sampling season was divided into 'Summertime' (May–October) and 'Wintertime' (November–April). The two periods were thoroughly chosen according to the special sunlight conditions during wintertime in countries located at latitudes greater than about 40°N [22]. In this study, we used the cutoff 25(OH)D < 50 nmol/L (20 ng/ml) to indicate 25(OH)D deficiency, 50–75 nmol/L (20–30 ng/ml) to indicate suboptimal levels and >75 nmol/L (>30 ng/ml) to indicate sufficient levels [23].

2.3. Research protocol

2.3.1. Rating scales

Current psychiatric symptoms were assessed using the Comprehensive Psychopathological Rating Scale (CPRS) [17], assessing the severity of reported and observed psychiatric symptoms. From the CPRS, the Montgomery-Åsberg Depression Rating Scale (MADRS) [17,24] was extracted. Suicidal ideation (SI) was assessed by the Suicide Assessment Scale (SUAS-S) [25].

2.3.2. SUAS suicide composite score

SUAS was developed by Stanley et al. and includes a total of 20 items. The scale covers five areas: emotional reactivity (items 4, 5 and 14), affect (items 1, 2, 9, 12 and 13), bodily states (items 3, 8 and 10), control and coping (items 6, 7, 11 and 15), and suicidal thoughts and behaviors (items 16–20). Item 16 refers to *suicidal thoughts*, item 17 to *purpose of suicide*, item 18 to *wish to die*, item 19 to *wish to live* and item 20 refers *to suicide plans*. Each of the items is rated on a five-point scale (0–4), with high scores indicating increasing severity [26,27]. The SUAS scale sum score has a range of 0–80 and the sum score of the five suicidal items (items 16–20) is 0–20. We calculated a suicide composite score, including the five suicide-related items (presented above), and used the median of the suicide composite score (6 points) to define *high-grade* suicidal ideation (hg-SI>6 points, $n = 100$) and *low-grade* suicidal ideation (lg-SI≤6 points, $n = 99$) [28].

2.4. Statistical procedures

Student's t-test and Chi-square test were used to compare group differences of confounding factors such as BMI, age (continuous variables) and sex, smoking, ethnicity, somatic illness, and sampling season (categorical variables). Pearson's Correlations were used to investigate relationship between 25(OH)D levels and continuous confounding factors.

Differences in mean values of 25(OH)D between patients and controls were tested with ANCOVA adjusting for sex, age, smoking, sampling season, ethnicity, somatic illness and BMI, and Chi-squared test was used to compare group differences in proportions. Binary logistic regression was used to model the probability of being patient or control (dependent variable) based on the 25(OH)D levels (independent variable) adjusting appropriate covariates. The independent covariates were selected *a priori* based on previous literature suggesting that they could be related to 25(OH)D levels [23]. In Model I, the results were adjusted for age, sex, BMI, smoking, somatic illness, ethnicity, and sample season. In Model II, only significant, or close to significant, covariates from Model I were included, i.e., BMI and somatic illness.

ANOVA with Bonferroni post hoc test was used to compare mean 25(OH)D values between different affective disorder diagnoses. Correlations were analyzed with Spearman's rho and significant correlations were evaluated with scatter plots; if linear relationships between variables were indicated, the results were further investigated with Pearson's correlation as well.

Normally distributed variables are presented with means and standard deviations (SDs), and variables not considered to be normally distributed are presented with medians and ranges (min–max). Normal distribution was examined by calculating kurtosis values divided by two standard errors; if the values were above one, it was considered as not normally distributed. Proportions are presented with percentages. The significance level was set to $p < 0.05$.

All statistical analyses were conducted using IBM SPSS Statistics 27 and R Studio 2021.09.0 (R v 4.1). Figures and visual statistics were created using the ggplot2 package v 3.3.5.

3. Results

3.1. Demographics and group comparisons

Demographic characteristics are summarized in Table 1. There were no significant differences between patients and healthy controls regarding sex, ethnicity, age, or blood sampling season. However, patients had a significantly higher BMI than controls (Student's t-test, $t = 2.79$; $p < 0.01$) and were more likely to be smokers than controls (Chi-squared test, $\chi^2 = 3.76$; $p = 0.05$). Women had significantly higher 25(OH)D levels compared to men (Student's t-test, $t = 3.37$; $p < 0.01$). Sixty percent of the patients had one or more somatic illness(es), among which muscular-skeletal system disorders (23%) and digestive system disorders (17%) were the most common (see Table 1). Age correlated significantly and positively with 25(OH)D (Pearson's $r = 0.19$, $p < 0.01$) and BMI correlated significantly and negatively with 25(OH)D (Pearson's $r = -0.16$, $p < 0.02$). There was a significant difference in 25(OH)D levels between winter and summer samples (mean levels 60.3 nmol/L and 70.3 nmol/L, respectively; Student's t-test, $t = 3.41$; $p < 0.01$). No significant differences in 25(OH)D mean levels were found between individuals who took psychotropic drugs, or anticonvulsant drugs in a separate analysis, and those who did not (Student's t-test, both $p > 0.08$), nor was there a difference in 25(OH)D mean levels between smokers and non-smokers (Student's t-test; $p = 0.84$). Individuals of Caucasian origin had significantly higher 25(OH)D levels than those with 'Other origin' (mean levels 66 nmol/L and 49 nmol/L, respectively; Student's t-test, $t = 2.06$; $p < 0.04$).

3.2. 25(OH)D levels in MDD and controls

Absolute levels of 25(OH)D are shown in Fig. 1A. Mean 25(OH)D levels in patient and healthy controls were 63.7 nmol/L (SD = 24.1) and 73.0 (SD = 17.8), respectively. The difference in 25(OH)D mean levels between patients and controls was significant after adjustment for sex, age, smoking, sampling season, ethnicity, somatic illness, and BMI (ANCOVA, $F = 4.89$, $p = 0.03$). Thirty percent of patients and 5% of

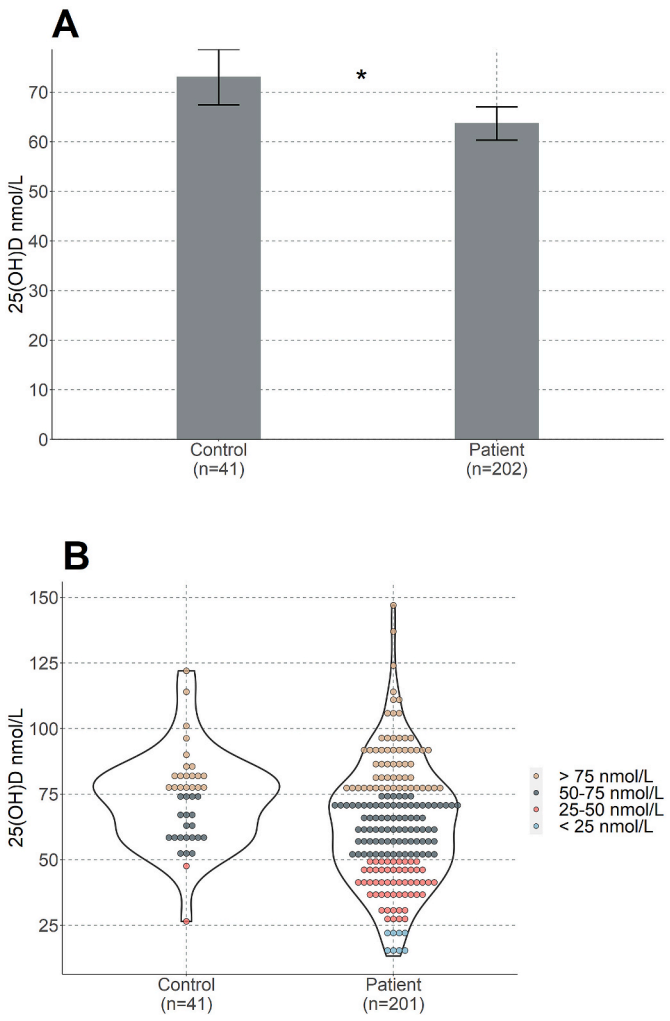

Fig. 1. **(A)** Bar graph showing mean levels of 25(OH)D in healthy controls and major depressive disorder (MDD) subjects. There were significantly lower levels of 25(OH)D in MDD subjects which remained significant after adjusting for sex, age, smoking, sampling season, somatic illness, and body mass index (BMI) (ANCOVA, F = 4.89, $p < 0.03$). **(B)** Violin Plot showing the distribution of 25 (OH)D levels in healthy controls and major depressive disorder (MDD) subjects divided into groups based on different 25(OH)D cut-offs. To gain greater visibility, one MDD subject with a 25(OH)D level of 174 nmol/L, was excluded in Fig. 1B. However, this MDD subject was included in statistical analysis. Error bars represent 95% CI. * Significant at the 0.05 level (2-tailed).

controls were 25(OH)D deficient (<50 nmol/L) (Chi-squared test, $\chi^2 = 11.38$; $p < 0.01$). Twenty-nine percent of the patients and 49% of controls had sufficient (>75 nmol/L) 25(OH)D levels (Chi-squared test, $\chi^2 = 5.95$; $p < 0.02$), see Fig. 1B.

Binary logistic regression showed significantly that the odds of being depressed decreased with approximately 17% for every increase of 10 nmol/L in 25(OH)D levels, see Table 2. We subsequently adjusted for potential confounders in Models 1 and 2, and the results were similar. In model I, all covariates and confounders chosen *a priori* were included. Due to the limited sample size of the controls, only three covariates/confounders could be included in the final model. Thus, in Model 2, we chose to include the covariates/confounders shown to be significant (or close to significant) in Model 1. Body mass index (BMI) was prioritized over smoking status since it has a stronger association with 25(OH)D than smoking status.

3.3. 25(OH)D levels in patients with different affective disorder diagnoses

There were no significant differences in mean 25(OH)D levels between the four affective disorder diagnoses groups (MDD single episode,

Table 3
Patient characteristics and group differences in the four different affective disorder diagnose groups.

Variables	MDD single episode ($n = 18$)	MDD recurrent episode ($n = 114$)	Chronic MDD ($n = 52$)	Dysthymia ($n = 18$)	*p*-value
Sex (% women)	44%	68%	65%	39%	0.04*
Age, years, (median (min-max))	37.2 (21–74)	38.4 (18–77)	38.5 (18–74)	36.3 (20–51)	0.93
BMI, mean (kg/m^2), (SD)	24.2 (5.3)	25.6 (4.3)	27.2 (5.9)	25.1 (4.6)	0.09
Smoker (% yes)	30%	20%	20%	18%	0.79
Sample season (% summer)	44%	45%	48%	56%	0.85
Somatic illness (% yes)	61%	65%	75%	28%	<0.01**
Psychotropic drugs (% yes)	78%	96%	100%	89%	<0.01**
Anticonvulsants (% yes)	0%	31%	23%	17%	0.03*
25(OH)D, mean (nmol/L), SD)	55.2 (22.5)	65.3 (25.2)	62.9 (24.6)	64.3 (15.2)	0.43
25(OH)D deficiency (% <50 nmol/L)	56%	25%	37%	22%	0.03*

* Significant at the 0.05 level (2-tailed).
** Significant at the 0.01 level (2-tailed).
Acronyms: major depressive disorder (MDD), body mass index (BMI), standard deviation (SD).

Table 2
Binary logistic regression, unadjusted and adjusted results, with MDD/controls as dependent variable.

Variables	Unadjusted results			Adjusted results[a]			Adjusted results[b]		
	OR	*p*-value	R^2	OR	*p*-value	R^2	OR	*p*-value	R^2
25(OH)D	0.849	0.02*	0.036	0.840	<0.05*	0.230	0.828	0.02*	0.179
BMI	1.142	<0.01**	0.065	1.106	0.07		1.109	0.05*	
Smoking	0.314	0.06	0.031	0.289	0.06		N/A	N/A	
Season	0.986	0.07	0.024	1.420	0.38		N/A	N/A	
Age	1.017	0.22	0.011	1.003	0.82		N/A	N/A	
Sex	1.590	0.23	0.011	1.665	0.23		N/A	N/A	
Somatic illness	0.263	<0.01**	0.096	0.263	<0.01**		0.258	<0.01**	

* Significant at the 0.05 level (2-tailed).
** Significant at the 0.01 level (2-tailed).
Acronyms: major depressive disorder (MDD), body mass index (BMI), odds ratio (OR).
[a] Model I: All variables possibly affecting dependent variable; BMI, smoking, sampling season, age, sex, somatic illness.
[b] Model II: Only significant variables, or close to significant, are included in the model.

MDD recurrent episode, chronic MDD and dysthymia) (ANOVA, $p = 0.43$), see Table 3. There were significant differences in the proportion of 25(OH)D deficient patients between the different affective disorder diagnoses, where the MDD single episode group had a significantly higher proportion of 25(OH)D deficient individuals than both the dysthymic group (Chi-squared test, $\chi^2 = 4.21$; $p = 0.04$) and the MDD recurrent group (Chi-squared test, $\chi^2 = 7.28$; $p < 0.01$) but not than the chronic MDD group (Chi-squared test, $p = 0.16$). No other proportion comparisons were significant between the different diagnose groups (all $p > 0.11$). There was no significant difference in mean 25(OH)D levels between patients with high-grade suicide ideation (hg-SI) and patients with low-grade suicide ideation (lg-SI) (Student's t-test, $p = 0.95$), nor in the proportion of 25(OH)D deficient patients between the hg-SI and the lg-SI group (Chi-squared test, $p = 0.65$).

3.4. Associations between 25(OH)D and symptom severity

There were no significant correlations between 25(OH)D and MADRS total score, SUAS total score or suicide composite score in all patients (Spearman's rho, all $p > 0.65$).

There were no significant correlations between 25(OH)D and symptom severity (MADRS, SUAS, suicide composite score) in patients with MDD single episode, MDD recurrent episode or chronic MDD (Spearman's rho, all $p > 0.26$). However, in patients with dysthymia, 25(OH)D correlated significantly and negatively with MADRS total score and SUAS-S total score, but not with suicide composite score (Spearman's rho $= -0.57$; $n = 18$; $p = 0.01$ and -0.76; $n = 15$; $p < 0.01$ respectively), see Fig. 2.

4. Discussion

In the present study we aimed to assess the relationship between 25(OH)D and different aspects of depressive disorders and suicidality in a large clinical cohort with depressed individuals with difficult-to-treat depression. To the best of our knowledge, only few studies have assessed 25(OH)D in different affective disorder diagnosis groups in a large clinical sample [4,29] or in relation to suicidality [9,30,31]. We found that depressed individuals overall had significantly lower 25(OH)D mean levels than healthy controls. In addition, we found some evidence for a link between 25(OH)D deficiency and different affective disorder diagnoses, since the MDD single episode group had a significantly higher proportion of 25(OH)D deficient individuals, although absolute levels of 25(OH)D did not differ significantly between the affective disorder diagnosis groups. We did not, as hypothesized, find any associations between 25(OH)D levels and suicidality. Although 25(OH)D was not directly associated with symptom severity in all depressed patients, significant correlations were found in those diagnosed with dysthymia.

In our study, clinically depressed individuals with insufficient treatment response had significantly lower 25(OH)D levels than healthy controls, which is in line with most, but not all, previous cross-sectional studies [3,4,32]. The reasons for discrepancies across studies may involve methodological issues such as small sample sizes, differences in inclusion criteria, different definitions of 25(OH)D deficiency and the possibility to adjust for relevant confounders [33–35]. Also, several studies used self-report questionnaires to assess a depressed state [36], while others have included patients with clinical depression diagnosis [4,37]. In a meta-analysis on the efficacy of 25(OH)D supplements in depression [38], Spedding et al. (2014) conducted sub-analyses of the included RCTs and found that studies investigating patients with clinically MDD diagnosis yielded positive results, whereas studies including depressed patients without a depressive disorder, or minimal non-clinically significant depressive disorder, yielded negative results. Another study, a well-designed, large cohort study with participants from the Netherlands Study of Depression and Anxiety (NESDA) [4], investigated the association between 25(OH)D and depressive disorders

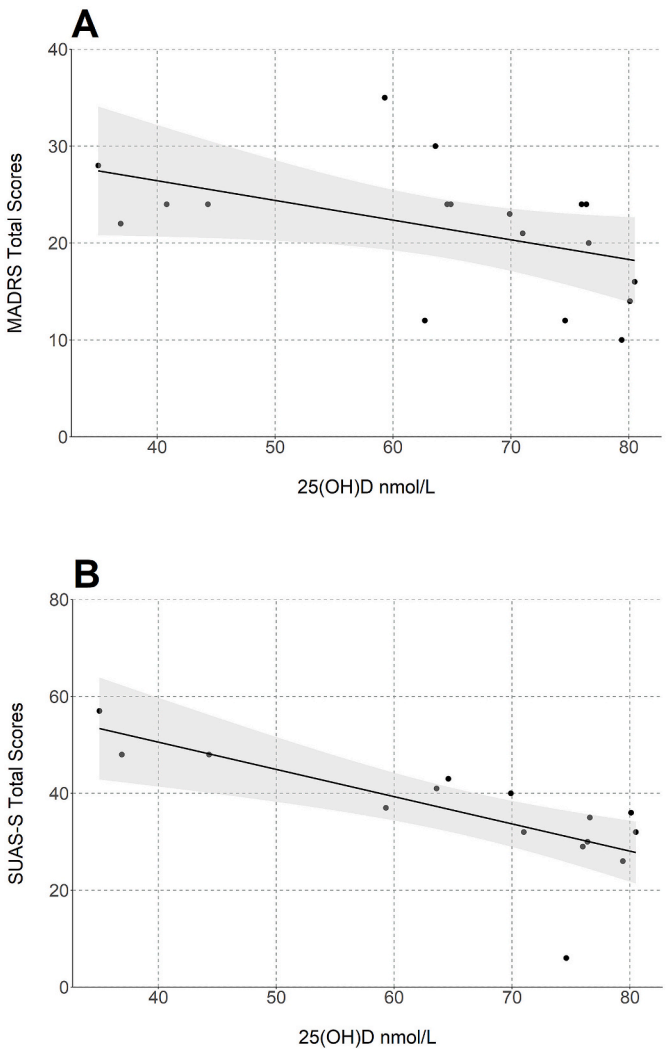

Fig. 2. Scatter plots showing correlations between levels of 25(OH)D and symptom severity (MADRS and SUAS-S total scores, respectively) in patients with dysthymia. **(A)** 25(OH)D correlated significantly and negatively with MADRS-S total score using parametric (Pearson's r $= -0.470$; $n = 18$; $p < 0.05$) and non-parametric test (Spearman's rho $= -0.57$; $p = 0.01$) **(B)** 25(OH)D correlated significantly and negatively with SUAS-S total score using parametric (Pearson's r $= -0.74$; $n = 15$; $p < 0.01$) and non-parametric test (Spearman's rho $= -0.76$; $p < 0.01$). Shaded area around regression line is the 95% CI.

(MDD and dysthymia, according to DSM-IV criteria) in 1102 individuals with current depressive disorder, 790 with remitted depressive disorder and 495 healthy controls [4]. They found that low 25(OH)D serum levels were associated with both the presence and the severity of depressive disorders in individuals with current depressive disorder, and the authors suggested that hypovitaminosis may represent an underlying biological vulnerability for depression. These findings, together with the results from our present study, imply that 25(OH)D may be involved in more severe cases of clinical depression, as opposed to sub-clinical depression in the general population.

There are many ways in which 25(OH)D could be one of the factors underlying the development, or the course, of depression. 25(OH)D exerts profound extra-skeletal effects in the body and the vitamin D receptor (VDR) is present in almost all bodily tissues, including the central nervous system [39,40]. Several downstream effects of 25(OH)D are associated with biological mechanisms previously suggested to be linked to psychiatric illness, e.g., the expression of neurotransmitters (for instance, dopamine and serotonin), stress response via the hypothalamic-pituitary-adrenal (HPA) axis, regulation of important

neurotrophic factors, as well as profound immunomodulatory effects [40–42].

As noted before, we did not find any significant differences in mean 25(OH)D levels between the four affective disorder diagnose groups in our present study, i.e., MDD single episode, MDD recurrent episode, chronic MDD and dysthymia. There are yet, to our knowledge, few studies exploring 25(OH)D in different affective disorder diagnose groups [29,37], or for that matter, *any* diagnose group of psychiatric disorders [6,43–46], and there is little evidence in the present literature of significant differences in 25(OH)D levels between diagnose groups of mental illness.

It is possible that it is not the actual 25(OH)D levels *per se* that may distinguish different diagnose groups of affective disorder, but rather certain downstream effects of 25(OH)D which could possibly differ between affective disorder diagnoses and subsequently affect the 25(OH)D levels and/or its association with depression symptom severity [15,40,42,47,48]. In the present study, we found a direct correlation between 25(OH)D and symptom severity in dysthymic patients, but not in any of the other depressive disorder diagnose group. Thus, it is possible that 25(OH)D levels have a more direct effect on the pathogenesis of symptoms in this specific patient group. A hypothesis would be that these differences derive from 25(OH)Ds' immunomodulatory effects, since, according to Ref. [47]; dysthymic patients may be distinguished from acute, episodic depression patients by a distinct immunological profile [47]. Future studies should investigate the hypothesis that 25(OH)D may play a more significant role in dysthymia than in other affective disorders, and they are also warranted to test the relationship between inflammation and 25(OH)D in different affective disorder diagnose groups.

Given the cross-sectional design of most studies to date, the underlying causes of 25(OH)D deficiency in some cases of depression are yet to be determined. For example, low levels of 25(OH)D might be caused by low outdoor activity as a consequence of the depressive disorder itself, i.e., due to lack of initiative and isolation. Our major source of 25(OH)D is sun exposure (UVB-radiation) to the skin, and thus, to avoid such confounder, both data on the *amount of time spent outdoors*, including *at what time-period during the day* - related to the specific latitude conditions - must be considered. These data are difficult to collect, and therefore, adjustment for sample season is the most common. Some studies have used the amount of physical activity as a proxy for time spent outdoors, with subsequent UVB-radiation exposure [4,8]. Milaneschi et al. also made an admirable attempt to handle the UVB-radiation confounder while adjusting their results for actual amount of sunlight hours in the 10 weeks preceding blood draw (measured using pyranometers at a weather station), instead of using sampling season. They also included data on degree of urbanization, which have been shown to affect 25(OH)D levels [49].

However, despite the important issue of time spent outdoors as a confounder to 25(OH)D levels in depressed individuals, the extra-skeletal biological effects of 25(OH) D suggested to be related to psychiatric illness [34,41], will still be manifested in 25(OH)D deficient individuals and could thus possibly interact with the development and/or symptom profile in depressed individuals.

Other important factors to consider in 25(OH)D studies related to psychiatric illness are female gender, smoking, and high BMI, which correlate to both lower 25(OH)D levels and depression [50–52]. In our study, we had the possibility to adjust for several relevant confounders and co-variates, e.g., age, sex, BMI, somatic illness, smoking status, ethnicity, and sampling season, and it did not change the significance of the results.

A limitation of the present study is its cross-sectional design, which makes it impossible to determine causality. The number of healthy controls ($n = 41$) was limited compared to the patient group ($n = 202$). We acknowledge that the negative results of the comparisons between the affective disorder diagnoses groups could possibly be type I error, as the number of patients with MDD single episode and dysthymia were of limited size (both groups $n = 18$). Most of the patients took one or more psychotropic medications, and more than half of the patients had one or more somatic illness. Another important limitation of our study is the lack of data regarding the actual amount of UVB-radiation exposure. However, we did adjust for sampling season which is also of high relevance.

The present study also has several strengths. Firstly, the patients were thoroughly investigated, and diagnosed according to DSM-IV, by either a psychiatric specialist or a resident in psychiatry with at least three years of psychiatric training under supervision from a senior colleague. The population consisted of 'real-life' psychiatric patients, a heterogenous population generally seeking psychiatric care, and the sample size of the study was relatively large (within the context), thus making it possible to perform subgroup analyses. Lastly, a significant strength of the study was the possibility to exclude patients who took 25(OH)D supplement.

5. Conclusions

The result of the present study indicates that 25(OH)D is associated with depression in individuals with difficult-to-treat depression. In our study, depressed individuals had significantly lower 25(OH)D levels than healthy controls and, additionally, 25(OH)D correlated negatively with symptom severity in dysthymic patients. Our results strengthen the current evidence of a presumed relationship between 25(OH)D and depression and highlight the dysthymic patient group to be an affective disorder diagnose group to pay extra attention to. To determine whether 25(OH)D supplementation might be of clinical value, either as a direct treatment of depressive symptoms or prophylactically to prevent the development of depressive disorders in 25(OH)D deficient individuals, well designed RCT's and longitudinal studies are highly warranted. Lastly, as 25(OH)D deficiency is over-represented in psychiatric patients, along with the importance of sufficient 25(OH)D levels for somatic health, patients with 25(OH)D deficiency are suggested to be treated to sufficient levels also to avoid possible negative consequences on mental health.

Author contributions

Cécile Grudet: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Roles/Writing - original draft, Writing - review & editing. Daniel Lindqvist: Funding acquisition, Investigation, Project administration, Supervision, Writing - review & editing. Johan Malm: Supervision, Writing - review & editing. Åsa Westrin: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing. Filip Ventorp: Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - review & editing.

Declaration of competing interest

None.

Acknowledgments

Daniel Lindqvist was supported by The Swedish Research Council (grant number 2020-01428), and state grants (ALF) from the province of Scania, Sweden. Åsa Westrin was supported by state grants (ALF) from the province of Scania, Sweden, and grants from the province of Scania.

None of the granting or funding agencies had a role in the design and conduct of the study; collection, management, analyzes and interpretation of the data; and preparation, review, or approval of the manuscript.

The authors would like to sincerely thank the psychiatric specialists

and residents in psychiatry who have been involved in the abundant data collection, especially Marie Asp, Amanda Holck, Johan Fernström and Livia Ambrus. Also, we would like to acknowledge the contribution from administrants and research nurses at the Science Center, Region Scania, in the recruitment process of patients, especially Johan Olsson and Linda Hansson. Lastly, we want to thank Simon Ventorp for the excessive work with data files and documentation.

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