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Published in:
Psychoneuroendocrinology

DOI:
10.1016/j.psyneuen.2014.08.016

2014

Psychoneuroendocrinology (2014) 50, 210—219

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Suicidal patients are deficient in vitamin D, associated with a pro-inflammatory status in the blood

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Received 6 June 2014; received in revised form 20 August 2014; accepted 25 August 2014

KEYWORDS
Suicidality;
Depression;
Cytokines;
Inflammation;
IL-1β;
IL-6;
TNF-α;
Th-1;
Th-2

Summary
Background: Low levels of vitamin D may play a role in psychiatric disorders, as cross-sectional studies show an association between vitamin D deficiency and depression, schizophrenia and psychotic symptoms. The underlying mechanisms are not well understood, although vitamin D is known to influence the immune system to promote a T helper (Th)-2 phenotype. At the same time, increased inflammation might be of importance in the pathophysiology of depression and suicide. We therefore hypothesized that suicidal patients would be deficient in vitamin D, which could be responsible for the inflammatory changes observed in these patients.
Methods: We compared vitamin D levels in suicide attempters ($n = 59$), non-suicidal depressed patients ($n = 17$) and healthy controls ($n = 14$). Subjects were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, and went through a structured interview by a specialist in psychiatry. $25(OH)D_2$ and $25(OH)D_3$ were measured in plasma using liquid-chromatography—mass-spectrometry (LC—MS). We further explored vitamin D's association with plasma IL-1β, IL-6 and TNF-α.
Results: Suicide attempters had significantly lower mean levels of vitamin D than depressed non-suicidal patients and healthy controls. 58 percent of the suicide attempters were vitamin D deficient according to clinical standard. Moreover, there was a significant negative association between vitamin D and pro-inflammatory cytokines in the psychiatric patients. Low vitamin D levels were associated with higher levels of the inflammatory cytokines IL-6 and IL-1β in the blood.

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http://dx.doi.org/10.1016/j.psyneuen.2014.08.016

Conclusion: The suicide attempters in our study were deficient in vitamin D. Our data also suggest that vitamin D deficiency could be a contributing factor to the elevated pro-inflammatory cytokines previously reported in suicidal patients. We propose that routine clinical testing of vitamin D levels could be beneficial in patients with suicidal symptoms, with subsequent supplementation in patients found to be deficient.

1. Introduction

The etiology of suicidal behavior is likely to be multifactorial, with genetic, environmental and psychological factors involved. Personality disorders (especially borderline personality disorder) and major depressive disorder are established risk factors for suicidal behavior. Among the underlying biological changes, accumulating studies indicate that a dysregulated immune system could be a contributing factor to depression, and possibly specifically to suicidality (Muller, 2014). Direct evidence of causality comes both from animal models, where induction of peripheral inflammation is known to lead to depressive changes (Bay-Richter et al., 2011), and from so called cytokine-induced depression in humans (Capuron et al., 2002), where treatment with interferons (IFN) of patients with hepatitis increases the risk for development of both depression and suicidality (Wichers and Maes, 2002). Post-mortem studies indicate that, irrespective of their primary psychiatric diagnosis, patients who die from suicide exhibit an increased level of microgliosis as a sign of neuroinflammation (Steiner et al., 2008). Other studies show elevated cytokine mRNA expression in the brains of suicide victims (Pandey et al., 2012; Tonelli et al., 2008). We previously demonstrated that patients who attempted suicide have elevated levels of the pro-inflammatory cytokine interleukin (IL)-6 in the cerebrospinal fluid (Lindqvist et al., 2009), and that suicidal patients have more pronounced inflammation in the blood than non-suicidal patients with depression (Janelidze et al., 2011). Confirming these results, O'Donovan et al. (2013) recently showed that suicidal ideation in depressive patients is specifically coupled to increased inflammation in the blood.

The pattern of inflammation in plasma of depressive and suicidal patients involves common pro-inflammatory cytokines such as IL-6, tumor necrosis factor (TNF)-α, and also the acute phase reactant C-reactive protein (CRP) (Dowlati et al., 2010). In most cases the origin of the observed low-grade inflammation is unknown. It could theoretically stem from many different sources, such as infectious agents, autoimmune reactions or stress (Zhang et al., 2012b; Liu et al., 2013). Low levels of vitamin D have also been suggested to contribute to different psychiatric disorders (Kjaergaard et al., 2011; Belvederi Murri et al., 2013). Interestingly, vitamin D exerts profound immuno-modulating effects (Borges et al., 2011). Several cross-sectional studies have shown a relation between low levels of vitamin D and more severe depressive symptoms (Milaneschi et al., 2014) and there is an over-representation of vitamin D deficiency among patients with psychiatric illnesses (McCue et al., 2012). Two recent prospective studies did indeed find that low vitamin D levels increase the risk for depression (Bertone-Johnson et al., 2011; Milaneschi et al., 2010). Also, a randomized double blind controlled trial showed that supplementation with vitamin D for one year had beneficial effects on depressive symptoms in overweight subjects (Jorde et al., 2008). However, the findings in the field are not consistent, which partly might be due to methodological differences.

The relationship of vitamin D deficiency and suicidality has been addressed in a small number of studies to date (Umhau et al., 2013; Tariq et al., 2011). Umhau et al. (2013) found that low vitamin D status is common in active duty service members, and the lowest levels were associated with an increased suicide risk. Tariq et al. (2011) hypothesized that vitamin D deficiency could be associated with increased risk of completed suicides and found an association of vitamin D deficiency with multiple suicide risk factors, such as chronic medical illnesses (autoimmune disorders, fibromyalgia and cancer), mood and anxiety disorders and schizophrenia. A seasonal pattern of suicides and attempts, with a peak incidence occurring in spring/early summer for men and a bi-modal peak in spring/early summer and autumn for women, is a well-known phenomenon in countries with a temperate climate (Christodoulou et al., 2012). These peaks cohere reasonably well with the seasonal variations of vitamin D levels in people living in respective countries (Rosecrans and Dohnal, 2014). For personality disorders, there is a lack of studies investigating the impact of vitamin D. One recent study did explore the relationship between vitamin D and basic personality traits, and indicated that vitamin D might promote an extrovert and open behavior. However, individuals with manifest Axis-I or Axis-II disorders were excluded (Ubbenhorst et al., 2011).

The effects of vitamin D on the immune system include a shift in the T helper (Th)-balance towards a Th2-phenotype, which in short can be said to result in a less pro-inflammatory state (van Etten and Mathieu, 2005). Since the immune dysregulation observed in depressed patients and suicide attempters include elevated levels of pro-inflammatory cytokines, we hypothesized that these patients suffer from an underlying vitamin D deficiency. We therefore compared the vitamin D levels between a group of suicide attempters, a group of non-suicidal depressed patients and a group of healthy controls and explored the relation between vitamin D and inflammatory cytokines in blood. We have previously established that the suicide attempters in this cohort have increased levels of IL-6 and TNF-α (Janelidze et al., 2011). In addition, we explored the vitamin D blood levels in the main groups of psychiatric diagnoses (Axis-I and Axis-II) as well as its' relation to specific aspects of suicidal behavior.

Table 1 Characteristics of the study participants regarding medical treatment and somatic conditions.

Variable	Suicide attempters ($n = 59$)	Non-suicidal depressed patients ($n = 17$)	Healthy controls ($n = 14$)
Medication (n)	SNRI (11) SSRI (10) Neuroleptics + SSRI (6) Anti-epileptics (6) Anti-epileptics + other (4) Lithium (3) Other combinations (9)		
Somatic illness[a] (n)	[b]Endocrine-nutritional and metabolic diseases (10)	[c]Endocrine-nutritional and metabolic diseases (2)	
	[d]Nervous system diseases (6)	[e]Nervous system diseases (2)	
	Eye diseases (1)		
	Ear diseases (1)		
	Circulatory system diseases (4)		
	Respiratory tract diseases (1)	Respiratory tract diseases (1)	Respiratory tract diseases (1)
	Digestive system diseases (3)	Digestive system diseases (1)	
	Skin diseases (1)	Skin diseases (1)	
	Musculoskeletal diseases (6)		
	[f]Pain conditions (3)		
	[g]Allergy (2)	[h]Allergy (1)	[i]Allergy (3)

[a] 10 of the suicide attempters, 1 of the non-suicidal depressed patients and 1 of the healthy controls had two or more somatic illnesses.
[b] 7 of the suicide attempters had an endocrine condition; 3 had diabetes and 4 had a thyroid disease.
[c] 1 of the non-suicidal depressed patients had an endocrine condition; Polycystic Ovarian Syndrome (PCO).
[d] Multiple Sclerosis (1), sequelae intoxication (1), migraine (2), fibromyalgia (1), neuropathy (1).
[e] Migraine (1), fibromyalgia (1).
[f] Post. op. fractures after suicide attempt (1), orifice of the stomach (1), pain NOS (1).
[g] Pollen allergics (1), nickel allergy (1).
[h] Allergy NOS (1).
[i] Cat, horse, pollen allergics, hard fruit (1), furred animal, hay fever (1), allergy NOS (1).

2. Subjects and methods

This study was approved by the Ethical Review Board for human studies, Lund/Malmö, Sweden (479/2006 and LU 82-01). All patients gave their written informed consent before enrollment. The research was performed according to the principles expressed in the declaration of Helsinki.

2.1. Subjects

Details about medication and somatic illness of the study participants are given in Table 1.

2.1.1. Suicide attempters

A total of 59 patients (25 men and 34 women) were enrolled on admission to Lund University Hospital after a suicide attempt. Mean age of the patients was 38 years (range 18—73, SD 14 years) and mean body mass index (BMI) 26 kg/m^2 (range 17-34, SD 4 kg/m^2). The patients were diagnosed according to the DSM-IV as: schizoaffective disorder ($n = 2$), psychotic disorder NOS ($n = 1$), major depressive disorder ($n = 10$), bipolar I disorder ($n = 3$), bipolar II disorder ($n = 12$), anxiety disorder NOS ($n = 4$), generalized anxiety disorder ($n = 1$), dysthymic disorder ($n = 4$), alcohol dependence ($n = 6$), substance dependence ($n = 2$), adjustment disorder ($n = 7$), adjustment disorder with depressed mood ($n = 3$) and depressive disorder NOS ($n = 3$). 29 of the suicide-attempters had an Axis-II disorder, 49 were treated with psychotropic drugs at the time for sample collection and 25 of the suicidal patients had a somatic diagnosis (Table 1).

2.1.2. Control group I: Non-suicidal depressed patients

17 non-suicidal patients (9 men and 8 women) with major depressive disorder were recruited from the Psychiatric Clinic, Lund University Hospital. Mean age of the patients were 35 years (range 22—54, SD 11 years) and mean BMI 25 kg/m^2 (range 18—54, SD 8 kg/m^2). The severity of the depression among the patients was a median MADRS total score of 25 (IQR: 23—31.5), which equals moderate depression severity. The non-suicidal depressed patients were

not treated with any psychotropic drugs for at least one month before sample collection. Seven of the non-suicidal depressed patients had a somatic diagnosis (Table 1).

2.1.3. Control group II: Healthy subjects

14 healthy controls subjects (7 men and 7 women) without any previous or ongoing psychiatric condition were randomly selected from the municipal population register in Lund. Mean age of the healthy subjects was 33 years (range 23—55, SD 11 years) and mean BMI 23 kg/m^2 (range 20—28, SD 3 kg/m^2). None of the healthy subjects were treated with psychotropic drugs. Three participants in the control group had a somatic condition (Table 1).

2.2. Clinical evaluation

All subjects went through a structured interview by a specialist in psychiatry and were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM IV) and the Structured Clinical Interview for DSM IV (SCID) I and II (American Psychiatric Association, 1994). A suicide attempt was defined as: 'Those situations in which a person has performed an actually or seemingly life threatening behavior with the intent of jeopardizing his/her life or to give the appearance of such intent, but which has not resulted in death' (Beck et al., 1972). The suicide attempts were classified into violent or non-violent based on the following criteria, as previously defined by Asberg et al. (1976) and Paykel and Rassaby (1978): non-violent suicid attempts include drug overdoses and single wrists cuts or a combination of these. All other attempts were classified as violent (e.g. hanging, drowning, suffocating, several deep cuts, poisoning, intentially thowing themselves into traffic, etc.) (Asberg et al., 1976; Paykel and Rassaby, 1978). All study participants underwent a general physical examination, which showed no evidence of ongoing infection or other unknown somatic condition.

2.3. Blood sampling

Blood samples from all subjects were collected between 7.30 and 8.00 in the morning, after a night of fasting. The suicidal patients were psychiatric inpatients, whereas the control-groups had spent the night at home. Samples from all study participants were collected within a week from the physical and psychiatric examination. The blood was placed on ice and centrifuged (3000 r/min, at +4 °C) within an hour. Plasma and serum were stored at −80 °C until analysis. Blood samples were collected from all seasons throughout the year for the suicidal patients and from all seasons, except for the summer, for the control groups. Blood samples from healthy controls and non-suicidal depressed patients were collected between 2001 and 2003 and from suicide attempters between 2006 and 2008.

2.4. Laboratory analyses

2.4.1. 25(OH)D_2 and 25(OH)D_3

Stored serum samples were thawed and analyzed for 25(OH)D_2, and 25(OH)D_3. The time of storage, 4—12 years at −80 °C, is not likely to have had an impact on the quality of the analysis due to the relatively stable vitamin D molecule (Wielders and Wijnberg, 2009; Antoniucci et al., 2005). The samples had not been thawed prior to analyses.

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 40 nmol/L and 5,0% at 120 nmol/L and for 25(OH)D_3 6,0% at 40 nmol/L and 4.0% at 120 nmol/L. The Department of Clinical Chemistry at Skåne University Hospital is accredited by SWEDAC (the Swedish Board for Accreditation and Conformity Assessment) and participates in the external quality assurance program of DEQAS (Vitamin D External Quality Assessment Scheme) (UK).

According to clinical guidelines (Phinney et al., 2012), in cases of a 25(OH)D_2 level >10 nmol/L, the 25(OH)D_2 level should be added to the 25(OH)D_3 level and accounted for as 25(OH)D_3 in the statistical analysis. However, no patients or controls in this study had a level of 25(OH)D_2 > 10 nmol/L and 25(OH)D_2 is therefore not included in the results presented here. The serum level of the inactive form of vitamin D, i.e. 25(OH)D_3, is referred to as 'vitamin D' throughout the article. Its active form, 1.25(OH)$_2$D$_3$, is referred to as 'active vitamin D'.

2.4.2. Cytokines

IL-6, IL-1β and TNF-α were measured in the plasma using ultra-sensitive electrochemiluminescence immunoassays according to the manufacturer's recommendations (MesoScale Discovery, UK). The method and absolute levels of the cytokines in these patients have been published (Janelidze et al., 2011). In the current paper we report the associations of the cytokines with vitamin D levels. All samples were analyzed in duplicates. The detection limits for IL-1β, IL-6 and TNF-α were 0.014 pg/mL, 0.050 and 0.075 pg/mL, respectively.

2.5. Statistical analyses

All statistical analyses were performed using the statistical package SPSS version 20 (SPSS Inc., Illinois, US). For power calculation, mean case concentration was predicted to 60 nmol/L and control concentration to 68 nmol/L based on previous literature (Milaneschi et al., 2014). Standard deviation was estimated to 25 nmol/L. The computed power for a two-tailed *t*-test showed that 87% power is achieved with $n = 59$. Vitamin D data displayed a normal distribution and all samples were above detection limit. Data for the cytokines were transformed into normal distribution using the natural logarithms before statistical analysis. Samples with zero cytokine levels were assigned a value corresponding to 1/10 of the lowest detectable sample in the assay. This applied to six samples of IL-1β, three from the non-suicidal depressed patients and three from the healthy controls. IL-6 and TNF-α had one sample each below the detection range, both occurring among the healthy controls. The potential effects of confounding factors were tested in linear regression models. Vitamin D was found to be associated with BMI and age of the subjects and therefore all analyses were performed correcting for these factors using linear regression.

For normally distributed variables, multiple group-wise comparisons were performed using One-way ANOVAs followed by Bonferroni-Dunn's post-hoc tests. Student's *T*-test was used in cases when only two groups were compared (e.g. violent vs non-violent suicide attempts). Mann—Whitney *U* test was used for non-normally distributed variables and groups of heterogeneous sizes. Fishers Exact Test was used for comparing proportions of patients with vitamin D deficiency between the groups. Correlations between cytokines and vitamin D were assessed using Pearson's *r*. The alpha-level of significance was set at $p < 0.05$.

3. Results

3.1. Effects of age, gender and BMI

Vitamin D correlated significantly with age and body mass index (BMI) (Pearson's *r*, 0.228 and −0.276, respectively, $p < 0.05$), but not did not differ between gender (Student's *T*-test, *ns*). All subsequent analyses are corrected for age and BMI.

3.2. Vitamin D levels are lowest in suicidal patients

The suicide attempters had significantly lower mean vitamin D levels than the non-suicidal depressed patients and the healthy controls (One-Way ANOVA, Bonferroni post-hoc test, $p < 0.05$) (Table 2, Fig. 1). The mean value of vitamin D among the suicide attempters was 47 ± 20 nmol/L whereas it was 62 ± 27 nmol/L among the depressed non-suicidal patients and 65 ± 26 nmol/L among the healthy controls.

58% of the suicide attempters in this study were found to have deficient levels of vitamin D according to clinical practice, i.e. below 50 nmol/L (Holick, 2007; Grober et al., 2013). The percentages of patients in the respective group with vitamin D deficiency are listed in Table 2. The group of suicidal patients contained a significantly greater portion of individuals with suboptimal or deficient vitamin D levels than the non-suicidal depressed patients and the healthy controls (Fisher's Exact Test, $p < 0.05$ for deficiency and $p < 0.01$ for suboptimal levels). No significant difference was found between the groups regarding the portions having a severe vitamin D deficiency (Fisher's Exact test, *ns*).

Table 2 Vitamin D mean levels and percent of the participants below different vitamin D cut off levels[a].

Variable	Suicide attempters ($n = 59$)	Non-suicidal depressed patients ($n = 17$)	Healthy controls ($n = 14$)
Vitamin D (mean ± SD), nmol/L	47 ± 20	62 ± 27	65 ± 26
% <25 nmol/L (10 ng/mL)	7%	12%	0%
% <50 nmol/L (20 ng/mL)	58%	29%	29%
% <75 nmol/L (30 ng/mL)	92%	59%	71%

[a] In clinical practice, the reference value for confirmed vitamin D deficiency is <50 nmol/L, and <25 nmol/L constitutes a severe deficiency. Values below 75 nmol/L are considered suboptimal.

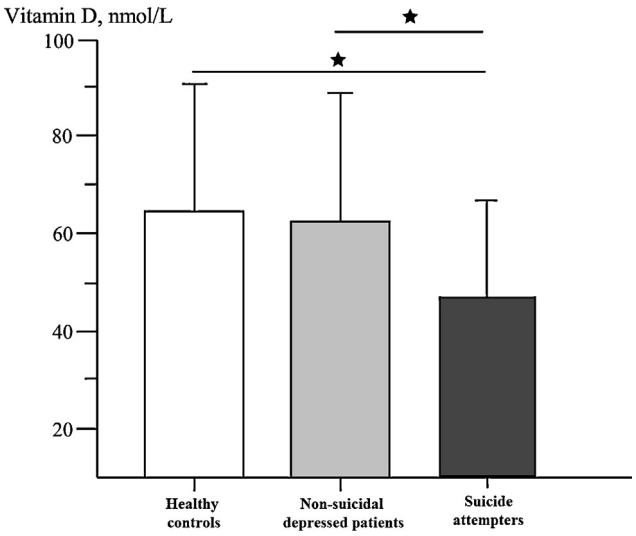

Fig. 1 Uncorrected mean vitamin D levels for the three studied groups. The significant differences in the mean vitamin D levels between the suicidal patients and the healthy controls/non-suicidal depressed patients were analyzed statistically using a variable corrected for age and BMI (* $p < 0.05$).

3.3. Seasonality

The samples from the suicide attempters were collected throughout all four seasons. There were no significant differences in the levels of vitamin D between samples collected in winter, fall, spring or summer (One-Way ANOVA, *ns*). The vitamin D levels were lowest in winter, but this did not reach statistical significance. The samples from the healthy controls and the depressive patients were collected in all seasons except for the summer, whereas seven samples from suicide attempters were collected in summer. To control for any potential effect of sampling season, we therefore re-ran the group comparisons excluding the samples from suicide attempters collected in the summer. This did not affect the significant group differences reported.

3.4. Effects of endocrine conditions and psychotropic medications on vitamin D levels

Eight of the study participants had an endocrine condition; seven of the suicidal patients and one of the non-suicidal depressed patients (Table 1). Exclusion of these eight participants did not affect the significant group differences reported.

Since the majority of suicidal patients were on psychotropic medication, we further performed a linear regression with vitamin D as dependent, and age, BMI and the major psychotropic medications (neuroleptics, Serotonin-Norepinephrine Reuptake Inhibitor (SNRI), Selective Serotonin Reuptake Inhibitor (SSRI), propiomazin and

benzodiazepines) as independent variables. None of the medications had any significant effect on the vitamin D levels ($n = 49$, linear regression, *ns*). Ten of the suicide attempters were treated with anti-epileptic drugs. No significant difference in the mean vitamin D levels between patients who took anti-epileptic drugs and patients who did not take anti-epileptic drugs were found (Mann—Whitney *U* test, *ns*).

3.5. Relationship of vitamin D with inflammatory cytokines

There was a negative correlation between vitamin D and IL-1β for all subjects ($n = 68$; Pearson's r: −0.240, $p < 0.05$). There were no significant correlations between vitamin D and IL-6 and TNF-alpha in all subjects (Pearson's *r*, *ns*).

Individual correlations in the three separate groups of participants showed a significant negative correlation between vitamin D and IL-1β in the group of suicide attempters ($n = 46$; Pearson's r: −0.321, $p < 0.05$). Vitamin D also correlated negatively with IL-6 in the group of non-suicidal depressed patients ($n = 17$; Pearson's r: −0.582, $p < 0.05$). No other associations were found between the cytokines and vitamin D in the individual groups.

3.6. Vitamin D, psychiatric diagnoses and suicidality

There was no significant difference between vitamin D levels in the three main groups of psychiatric diagnosis, i.e. major depressive disorder ($n = 10$), bipolar disorder ($n = 15$) and other psychiatric illnesses ($n = 28$) (One-Way ANOVA, *ns*) or between those with an affective disorder ($n = 31$) and those without an affective disorder ($n = 22$) (Mann—Whitney's *U*-test, *ns*). Likewise, there was no significant difference between subjects with an Axis-II disorder ($n = 27$) compared to those without ($n = 26$) (Student's *T*-test, *ns*). The levels of vitamin D and the details of the respective groups are shown in Table 3.

We also analyzed different aspects of suicidality by comparing suicide attempt repeaters ($n = 35$) to non repeaters ($n = 17$); and those who had committed a violent suicide attempt ($n = 6$) to those who had committed a non-violent suicide attempt ($n = 46$) but there were no significant differences between these groups (Mann—Whitney's *U*-test, *ns*) (Table 3).

Table 3 Vitamin D mean levels in different groupings of the suicidal patients.

Groups	(*n*)	Vitamin D level (mean ± SD) (nmol/L)
Major depressive disorder	10	47 ± 18
Bipolar disorder	15	49 ± 19
Other psychiatric illnessses	33	47 ± 22
[a]Affective disorder	32	50 ± 20
[b]Non-affective disorder	26	44 ± 20
Axis-II disorder	29	47 ± 22
No Axis-II disorder	30	47 ± 19
Repeater	38	49 ± 21
Non-repeater	20	42 ± 19
Violent suicide	6	45 ± 19
Non-violent suicide	52	47 ± 21

[a] The affective disorder group contains patients with major depressive disorder ($n = 10$), bipolar I and II disorder ($n = 15$), dysthymic disorder ($n = 4$) and depressive disorder NOS ($n = 3$).
[b] The non-affective disorder group contains patients with schizoaffective disorder ($n = 2$), psychotic disorder NOS ($n = 1$), anxiety disorder NOS ($n = 4$), generelized anxiety disorder ($n = 1$), alcohol dependence ($n = 6$), substance dependence ($n = 2$), adjustment disorder ($n = 7$) and adjustment disorders with depressed mood ($n = 3$).

4. Discussion

We found that suicidal patients had significantly lower mean levels of vitamin D than both non-suicidal depressive patients and healthy controls. 58% of the suicidal patients were clinically deficient in vitamin D, compared to around 30% in the healthy controls and the non-suicidal depressed patients. Interestingly, there was a negative association between the blood levels of vitamin D and pro-inflammatory cytokine levels in the psychiatric patients. Thus, the lower the vitamin D, the higher the levels of the key inflammatory cytokines IL-6 and IL-1β in the blood. The design of this study focused on suicidality as a cross-diagnostic phenomenon, enrolling patients based on a suicide attempt, irrespective of underlying psychiatric diagnosis. Our hypothesis was that we would find significant biological changes associated with suicidality. Indeed, in this study we found that the suicide attempters had significantly lower vitamin D levels than both the non-suicidal depressive patients and the healthy controls. In a previous study, we found similar results for elevations of pro-inflammatory cytokines, where IL-6 and TNF-α were increased in the suicidal patients, but not in non-suicidal depressive and healthy controls (Janelidze et al., 2011). Therefore, our results indicate that these biological alterations might indeed be specific, or most pronounced in suicidal patients. To further analyze whether the vitamin D levels differed between diagnostic groups, we here compared the levels between patients with and without personality disorders as well as the main Axis-I diagnoses among the suicide attempters, but we did not detect any significant differences related to suicidality apart from those described above. In the future, it will be of interest to compare the vitamin D levels between other groups of psychiatric patients.

The cross-sectional design of this study does not allow any conclusions regarding whether vitamin D is a direct biological cause of psychiatric illness or suicidal behavior. However, there are several ways by which vitamin D could affect the brain. For example, vitamin D receptors (VDRs) and the vitamin D activating enzyme 1α-hydroxylase (1αOHase) are widely spread in the adult human brain, suggesting an autocrine/paracrine function of vitamin D (Eyles et al., 2005). Both VDRs and 1αOHase have been identified in neurons and microglial cells, the primary mediators of a pro-inflammatory immune response in the brain (McCann and Ames, 2008). Brain areas with abundant VDRs are Prefrontal cortex, Cingulate gyrus, Hippocampus (especially

CA1 and CA2), Substantia Nigra and Hypothalamus. Vitamin D has been proposed to act like other neurosteroids and play a role in maintaining normal brain function by modulating neuronal excitability, regulating the production of specific neurotrophins and by having neuroprotective effects (Eyles et al., 2005; Harms et al., 2011). Animal models have shown that active vitamin D also increases the first and rate-limiting enzyme, tyrosine hydroxylase (TH), in the catecholamine biosynthetic pathway, and thus may contribute to responses and adaptation to stress, arousal, mood and motivated behavior (Puchacz et al., 1996).

Vitamin D is also known to exert profound immune modulating effects by increasing the levels of anti-inflammatory cytokines such as IL-10, IL-4, IL-5 and transforming growth factor (TGF)-β, and by decreasing pro-inflammatory cytokines IL-1β, IL-2, IL-6, INF-γ, TNF-α and IL-12 (Baeke et al., 2010; Zhang et al., 2012a). The net result of these effects is a shift from a Th1 to a Th2 immunological phenotype, which is considered to be a less pro-inflammatory state (Borges et al., 2011). As we and others have previously found pro-inflammatory cytokines to be increased in suicidal patients (Lindqvist et al., 2009; Janelidze et al., 2011), our hypothesis was that a deficiency in vitamin D could be part of the pathology underlying these changes. Indeed, because we here found that low vitamin D levels were significantly associated with higher levels of the pro-inflammatory cytokines in plasma of the psychiatric patients, the data is in line with our primary hypothesis.

Another way in which vitamin D could be a contributing factor to suicidality is its impact on the serotonin levels. Low serotonin levels have, for example, been associated with aggressive suicide attempts and impulsive behavior, characteristic for suicidal patients (Sadkowski et al., 2013). Active vitamin D has been shown to activate the transcription of the serotonin-synthesizing gene, tryptophan hydroxylase 2 (TPH2), in the brain (Patrick and Ames, 2014). Active vitamin D has also been shown to increase p11 (also known as S100A10), a protein which interacts with and transports the 5-hydroxytryptamine (5-HT)$_{1B}$ receptor (serotonin receptor) from the cytoplasm to the membrane (van de Graaf et al., 2003; Zhang et al., 2011). A recent study demonstated that p11 mRNA expression levels are lower in the stress-related brain regions of suicide victims (Anisman et al., 2008).

A deficiency in vitamin D is defined as blood levels of less than 50 nmol/L, while levels below 25 nmol/L are considered to constitute a severe deficiency. Vitamin D levels between 100 and 150 nmol/L are considered ideal, but levels above 75 nmol/L are generally thought to be sufficient (Holick, 2007; Grober et al., 2013). While most of the recommendations concerning the clinical reference interval for vitamin D are based on skeletal health, the effects on other biological systems are less characterized, and it is possible that different vitamin D levels are optimal in different tissues. Vitamin D suppresses lipopolysaccharide (LPS)-induced IL-6 and TNF-α production by human monocytes in culture at vitamin D levels of 75 nmol/L, but not at 37.5 nmol/L. It is therefore proposed that patients with chronic inflammatory diseases, deficient in vitamin D, should benefit from vitamin D supplementation up to a serum level of above 75 nmol/L (Zhang et al., 2012a). Multiple sclerosis (MS) has also been linked to vitamin D deficiency. In an animal model of MS, experimental autoimmune encephalomyelitis (EAE), vitamin D status determines the severity of the disease (Cantorna, 2000; Ascherio et al., 2014). Thus, supplementation with active vitamin D blocks EAE-progression whereas vitamin D deficiency accelerates the EAE onset. Recent clinical guidelines now recommend that patients with MS who are deficient in vitamin D should receive supplement of the vitamin when their blood levels are below 75 nmol/L (Brum et al., 2014). Interestingly, in our current study as many as >90% of the suicide attempters had suboptimal vitamin D levels (below 75 nmol/L), and could thus be candidates for vitamin D treatment in future trials. Thus, we argue that routine clinical testing of vitamin D levels could be beneficial in psychiatric patients (specifically in patients with suicidal symptoms), with subsequent supplementation in patients that are found to be deficient, or with suboptimal levels.

Why were the subjects in our study deficient in vitamin D? Although the study did not aim to characterize the underlying cause of vitamin deficiency, we were able to examine the effects of some potential confounders. Well-established risk factors for vitamin D deficiency are high age, obesity, liver or kidney disease and low exposure to sunlight. In our study, all results were corrected for effects of age and BMI. None of the study participants had any liver or kidney disease. However, a limitation of the study is that we do not have any detailed data regarding the ethnicity of the participants, although the large majority of the patients at Lund's University Hospital are of Swedish origin with a Caucasian ethnicity. We also lack data regarding smoking habits among the participants, which is a potential confounder that could influence cytokine levels and possibly also vitamin D levels (Brot et al., 1999; Shirazi et al., 2013). Another potential confounder is the influence of sun exposure. As the major source of vitamin D production is the skin, vitamin D status can be considered a surrogate marker of sunlight exposure. It has been demonstrated, in vitro and in vivo, that UV-light mediates both inflammatory and immunosuppresive effects that may be at least partially distinct from those exerted directly by vitamin D (Norval and Halliday, 2011; Li et al., 2013). Clinical studies investigating the effects of UV-therapy on depressive and suicidal patients are therefore warranted independently of studies aimed solely at restoring vitamin D levels. We do not have any data about the sun exposure of the study participants prior to the study and can therefore not adjust our results for this confounder, although we could conclude that seasonality did not impact the group differences.

There is currently insufficient knowledge about the effects of different pharmacological agents on vitamin D. According to a recent review there is insufficient evidence to determine whether medications that have previously been suggested to alter vitamin D levels actually do have this effect (Robien et al., 2013). It is well known, though, that osteomalacia is a serious side effect from certain drug therapies. Chronic administration of anti-epileptic drugs and treatment with the antimicrobial drug rifampin have shown the strongest association with osteomalacia. The exact mechanism underlying this association is yet not fully understood, but lowered vitamin D levels is one of the proposed mechanisms (Wang et al., 2013). In our study, none of the major pharmacological groups of agents had any significant effects on the levels of vitamin D. Ten of the suicide attempters were treated with anti-epileptic drugs, although there were no significant difference in the mean vitamin D

levels between them and the patients who did not take antiepileptic drugs. We therefore do not interpret our data as resulting from any medications used by the patients.

5. Conclusions

In summary, we found that 58% of the suicide attempters in our study suffered from vitamin D deficiency, and that their levels of vitamin D were significantly lower than those in healthy controls and depressive patients without suicidality. Interestingly, the low levels of vitamin D in plasma were associated with elevated inflammatory markers in the psychiatric patients (specifically IL-1β and IL-6). As we and others have previously shown that peripheral and central inflammation is increased in suicidal patients, we here suggest that low levels of vitamin D could be a contributing cause of this inflammation. As inflammation is suggested to directly be part of the neural mechanisms underlying depressive and suicidal behavior, it should be of high relevance to detect and cure the vitamin D deficiency in these patients. As blood tests for vitamin D levels can be readily done, as well as substitution with vitamin D, this could be implicated in clinical practice within a short time-frame. To determine whether vitamin D substitution decreases systemic inflammation and improves depressive and suicidal symptoms, clinical trials are highly warranted.

Role of funding source

Funding body agreements and policies.

Conflicts of interest statement

All authors declare that there are no conflicts of interests that could inappropriately influence, or be perceived to influence, their work.

Contributors

Cécile Grudet, Lena Brundin, Åsa Westrin and Johan Malm designed the study and interpreted the data. Cécile Grudet managed the literature searches, performed statistical analysis and prepared the main draft of the manuscript. Lena Brundin was responsible for cytokine measures, statistical analysis and main manuscript writing. Åsa Westrin was responsible for patient recruitment and examination of the patients. Johan Malm carried out the vitamin D analyses. All authors contributed to manuscript writing and have approved of the final version of the manuscript.

Acknowledgments

This study was supported by Michigan State University and Van Andel Research Institute (LB), the Swedish Research Council (VR) No. 2009-4284 and 2011-4787 (LB), the Province of Scania Clinical State grants (ALF, for AW, JM and LB), Fundacion Federico SA (JM). The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. We are grateful to Dr Shorena Janelidze for analyzing cytokine levels in the patients, and to Professor Lil Traskman-Bendz and Dr. Charlotta Sunnqvist for their role in recruiting patients and designing the study.

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