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ORIGINAL ARTICLE

Vitamin D deficiency is associated with acute ischemic stroke, C-reactive protein, and short-term outcome

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Abstract The aim of this study was to investigate whether vitamin D deficiency (VDD) is associated with acute ischemic stroke, inflammatory markers, and short-term outcome. 168 acute ischemic stroke patients and 118 controls were included. The modified Rankin Scale (mRS) was applied up to 8 h of admission (baseline) and after three-months follow-up, and blood samples were obtained up to 24 h of admission to evaluate serum levels of 25-hydroxivitamin D [25(OH)D] and inflammatory markers. Vitamin D levels classified the individuals in sufficient (VDS ≥ 30.0 ng/mL), insufficient (VDI 20.0–29.9 ng/mL), and deficient (VDD < 20.0 ng/mL) status. Patients had lower levels of 25(OH)D, higher frequency of VDD (43.45% vs. 5.08%, OR: 16.64, 95% CI: 5.66–42.92, $p < 0.001$), and higher inflammatory markers than controls ($p < 0.05$). Patients with VDD showed increased high sensitivity C-reactive protein (hsCRP) levels than those with VDS status ($p = 0.043$); those with poor outcome presented with lower 25(OH)D levels than those with good outcome ($p = 0.008$); moreover, 25(OH)D levels were negatively correlated with mRS after three-months follow-up ($r = -0.239$, $p = 0.005$). The associations between VDD and higher hsCRP levels and between 25(OH)D levels and poor outcome at short-term in acute ischemic stroke patients suggest the important role of vitamin D in the inflammatory response and pathophysiology of this ischemic event.

Keywords Stroke · Vitamin D · Inflammation · C-reactive protein · Vitamin D deficiency

Introduction

Stroke is sudden onset of focal neurological deficit and a major cause of disability and mortality worldwide (Sacco et al. 2013). Acute ischemic stroke has a heterogeneous etiology caused by unmodifiable risk factors, such as genetic, age, and sex, as well as by modifiable risk factors including hypertension, diabetes mellitus (DM), dyslipidemia, sedentary lifestyle, and smoking (Mozaffarian et al. 2015). Cerebral ischemic injury is associated with the induction of several inflammatory events, including the infiltration of circulating immune cells and activation of microglia, astrocytes, and endothelial cells (Ladeby et al. 2005).

Previous studies have reported that vitamin D deficiency is associated with cardiovascular disease events and mortality (Kendrick et al. 2009; Durup et al. 2015). VDD has been associated with increased prevalence of individual cardiovascular risk factors, such as endothelial dysfunction (Tarcin et al. 2009), DM (Mitri et al. 2011), dyslipidemia (Zittermann et al. 2011), and hypertension (Martins et al. 2007), and is considered an independent risk factor for the occurrence of acute ischemic stroke (Marniemi et al. 2005; Pilz et al. 2008; Sun et al. 2012; Brøndum-Jacobsen et al. 2013; Chaudhuri et al. 2014). Moreover, hypovitaminosis D was independently

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associated with larger ischemic infarct volume (Tu et al. 2014; Turetsky et al. 2015) and poor outcome (Park et al. 2015).

Bone and skeletal muscle, brain, prostate, breast and colon tissues as well as immune cells have a vitamin D receptor (VDR) and respond to the active form 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] (Holick 2007). Therefore, apart from its classical effects on bone and skeletal homeostasis (Ebeling 2014), vitamin D has been associated with the regulation of inflammation through several mechanisms, including inhibition of prostaglandin, mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) pathways (Cohen-Lahav et al. 2006; Liu et al. 2014; Huang et al. 2015), down regulation of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12, and interferon (IFN)-γ, and up regulation of anti-inflammatory T regulatory (Treg) and Th2 cells and their cytokines (Boonstra et al. 2001; Penna et al. 2005; Zhang et al. 2012). All these potential mechanisms may explain the link between vitamin D with cardiovascular diseases and stroke.

The association between vitamin D and acute ischemic stroke has been evaluated in different populations; however, conflicting results were found. While some studies reported an association between low levels of vitamin D and stroke (Marniemi et al. 2005; Sun et al. 2012; Michos et al. 2012; Brøndum-Jacobsen et al. 2013; Chaudhuri et al. 2014; Tu et al. 2014; Park et al. 2015), as well as its relationship with outcome and prognosis (Brøndum-Jacobsen et al. 2013; Daubail et al. 2013, 2014; Wang et al. 2014; Tu et al. 2014; Turetsky et al. 2015; Park et al. 2015), others have not found such associations (Bolland et al. 2010; Drechsler et al. 2010; Kühn et al. 2013; Gupta et al. 2014; Majumdar et al. 2015). Moreover, only two of these previous studies evaluated the association of vitamin D and inflammatory markers in patients with acute ischemic stroke (Tu et al. 2014; Park et al. 2015) and also showed conflicting results. To clarify this issue, the aim of the present study was to evaluate the association between vitamin D levels and acute ischemic stroke, as well as with inflammatory markers and short-term outcome in these patients.

Materials and methods

Study subjects

The protocol was approved by the Institutional Research Ethic Committee of the State University of Londrina, Paraná State, Brazil (CAAE 0250.0.268.000-11) and a written consent form was obtained from all of the individuals. A total of 168 acute ischemic stroke patients diagnosed with focal neurological signs or symptoms of vascular origin that persisted for >24 h, confirmed by brain computed tomography (CT) and clinic examination were consecutively recruited from January 2013 to January 2015 from the Emergency Room of the University Hospital of State University of Londrina. The acute ischemic stroke subtypes were classified according to the TOAST criteria (Adams et al. 1993). As controls, 118 individuals from the general population of Londrina, controlled for age, sex, ethnicity, and body mass index (BMI), and with no history of stroke or myocardial infarction were enrolled in the same period. Patients and controls with fever, acute infections, hematological, inflammatory or autoimmune diseases, with renal or liver failure, cancer, cerebral hemorrhage, and those using calcium and/or vitamin D supplements were excluded. Demographic, epidemiological, anthropometric, and clinical data including traditional stroke risk factors and the use of any therapeutic drug were obtained using a standard questionnaire at the admission of the individuals. Anthropometric measures were verified by body weight and height reported by the individuals, when it was possible, or by the patient's family. BMI was calculated as weight (kg) divided by height (m) squared. The ethnicity was self-reported as Caucasian and non-Caucasian (Asiatic, Black, and Afro-Brazilian) (Brazil 2011).

Baseline blood pressure evaluations were obtained at the admission of the individuals (patients and controls) using digital apparatus properly calibrated, and the mean of three measurements was used in the analysis. The use of antihypertensive medication was an indication of hypertension (James et al. 2014); DM was defined as a fasting serum glucose ≥ 126 mg/dL, a non-fasting serum glucose ≥ 200 mg/dL and/or use of anti-diabetic medication (American Diabetes Association 2014); dyslipidemia was defined by the presence of one or more than one of the abnormal serum lipid concentration: total cholesterol ≥ 200 mg/dL, low-density lipoprotein cholesterol ≥ 130 mg/dL; high-density lipoprotein cholesterol < 40 mg/dL, triglycerides > 150 mg/dL [National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation 2002]. The functional impairment was evaluated using the modified Rankin Scale (mRS) (Bonita and Beaglehole 1988) applied within the first 24 h of admission (baseline) and the values were used to categorize the patients with mild (mRS < 3) or moderate/severe functional impairment (mRS ≥ 3). The mRS was also applied after three-months follow-up through clinical examination or using telephone interviews with the patients or their relatives (Wang et al. 2014) and the values were used to categorize the patients with good (mRS < 3) or poor outcome (mRS ≥ 3) (Park et al. 2015).

Vitamin D and inflammatory markers

Peripheral blood samples were obtained under non-fasting state, with and without EDTA as anticoagulant. From the stroke patients, the samples were obtained within the first 24 h of admission in the hospital; from controls, the

samples were obtained at the time of inclusion in the study. Plasma and serum samples were immediately separated by centrifugation (2,500 rpm for 15 min) and stored in aliquots at −80 °C until analyzes. Serum levels of 25 dihydroxyvitamin D [25(OH)D] were determined using chemiluminescent microparticle immunoassay (CMIA) (Architect, Abbott Laboratory, Abbott Park, IL, USA). Vitamin D sufficiency (VDS) was defined with values ≥ 30.0 ng/mL, vitamin D insufficiency (VDI) was defined with values from 20.0 to 29.9 ng/mL and vitamin D deficiency (VDD) with values < 20.0 ng/mL (Holick 2007). Plasma levels of TNF-α, IL-6, and IL-10 were evaluated using a sandwich enzyme-linked immunosorbent assay (ELISA, eBioscience, San Diego, California, USA). White blood cell, platelet counts and erythrocyte sedimentation rate (ESR) were determined using hematological autoanalyzers. Serum levels of high sensitivity C-reactive protein (hsCRP) and ferritin were determined using CMIA (Architect, Abbott Laboratory, Abbott Park, IL, USA).

Statistical analysis

Analyses of contingency tables (χ^2 test) were employed to check the associations between categorical variables and diagnostic groups. We assessed the differences in continuous variables between groups using analysis of variance (ANOVAs) followed by the Tukey test to examine multiple comparisons among subgroup means, when appropriated. Categorical variables were expressed as absolute number (n) and percentage (%) and continuous variables were expressed as mean ± error standard of mean (SEM). The mRS values were expressed as median and interquartile range (IQR) of 25–75%. The Kolmogorov-Smirnov test was used to assess normality of distribution. Logarithmic (Ln) transformation of continuous data was used in the analysis when the variables were not normally distributed or when there was heterogeneity of variance (as assessed with the Levene test) and rechecked the assumptions for parametric analysis. Bivariate logistic regression analysis was used to define the significant predictor, with odds ratio (OR) and 95% confidence intervals (CI) of stroke patients versus controls using the markers and other significant characteristic data, in six different models. Further, the significant variables were also evaluated using Bonferroni post-test. Multinomial logistic regression analysis was used to define the significant associations, with OR and 95% CI of vitamin D status in acute ischemic stroke using the variables with p value < 0.10. Correlations between the serum levels of 25(OH)D and mRS after three-months follow-up were performed by Pearson's correlation. The statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA) and significance was defined as $p < 0.05$.

Results

Table 1 shows the baseline characteristics and vitamin D status in patients and controls. As expected, patients and controls did not differ in the controlled variables, such as sex, age, ethnicity, and BMI. Most patients were men (96/57.1%), Caucasians (132/78.6%) and the mean age (±SEM) was 67.9 ± 1.0 years. Patients had higher frequency of smoking ($p < 0.001$), DM ($p = 0.025$), and VDD than controls ($p < 0.001$). Seventy-three (43.5%) patients showed VDD, whereas it occurred in only 6 (5.08%) controls ($p < 0.001$). The mean (±SEM) of 25(OH)D was 22.54 ng/mL (0.82) in patients and 30.37 ng/mL (0.80) in controls ($p < 0.001$). The outcome of ANOVAs performed in the different inflammatory markers demonstrated higher ESR ($p = 0.002$), total peripheral leukocyte counts ($p < 0.001$), hsCRP ($p < 0.001$), ferritin ($p = 0.002$), IL-6 ($p < 0.001$), and TNF-α ($p = 0.014$) in the patients compared with controls.

Among stroke subtypes, 58 (34.5%) patients had large artery atherosclerosis stroke (LAAS), 53 (31.6%) lacunar infarct (LAC), 26 (15.5%) cardio-embolic infarct (CEI), 5 (3.0%) other determined etiology (ODE), and 26 (15.5%) had undetermined etiology (UDE). Moderate/severe functional impairment in admission was found in 124 (79.0%) patients with a median (IQR) mRS score of 4.00 (3.00–5.00), and after three-months follow-up, 90 (67.2%) patients had poor outcome, with a median (IQR) mRS of 4.00 (5.00–2.00) (data not shown).

Further, some results of the univariate statistical analysis were used to delineate the significant explanatory variables as determinants of independent association between vitamin D status and diagnostic groups in subsequent models of logistic regression analysis (Table 2). With model 1, controlled for age, sex, BMI, and ethnicity, VDD remained associated with acute ischemic stroke (OR 16.32, 95% CI: 5.89–45.17; $p < 0.001$); with model 2, that included model 1 and smoking status, this association remained significant (OR 16.07, 95% CI: 5.77–44.70; $p < 0.001$); when DM was added into model 3, the association was also significant (OR 14.60, 95% CI: 5.21–40.23; $p < 0.001$); with model 4, that included dyslipidemia, the association also remained significant (OR 14.44, 95% CI: 5.15–40.53; $p < 0.001$). Finally, with model 5, after additional adjustment for drugs, including use of antihypertensive, hypoglycemic, and hypolipemiant medication, the VDD remained associated with acute ischemic stroke (OR 16.64, 95% CI: 5.66–42.92; $p < 0.001$).

The characteristics of the patients stratified by their vitamin D status are summarized in Table 3. Patients with VDD were more likely to be female, older, and with higher mRS at baseline than those with VDI and VDS ($p = 0.003$, $p = 0.008$, and $p = 0.047$, respectively). However, there were no statistically significant differences between stroke subtypes in relation to vitamin D status ($p = 0.861$). Furthermore, sex, age, and mRS were evaluated using Bonferroni post-test and these variables remained associated with VDD.

Table 1 Baseline characteristics and vitamin D status of acute ischemic stroke patients and controls

Characteristics	Controls ($n = 118$)	Stroke Patients ($n = 168$)	p-value
Age (years)	65.16 (±1.12)	67.87 (±1.03)	0.080
Sex			
Male	57 (48.30)	96 (57.14)	0.140
Female	61 (51.69)	72 (42.86)	
Ethnicity			
Caucasian	97 (82.20)	132 (78.57)	0.449
Non Caucasian	21 (17.80)	36 (21.43)	
BMI (kg/m^2)†	27.26 (±0.41)	26.42 (±0.49)	0.085
Diabetes mellitus	30 (25.42)	64 (38.10)	0.025
Dyslipidemia	41 (34.74)	74 (44.04)	0.221
Smoking	8 (6.78)	39 (23.21)	<0.001
Antihypertensive	51 (43.22)	119 (70.83)	<0.001
Hypoglycemic	20 (16.95)	42 (25.00)	0.065
Hypolipemiant	25 (21.19)	45 (26.79)	0.188
Vitamin D (ng/mL)	30.37 (±0.80)	22.54 (±0.82)	<0.001
Vitamin D status			
Deficient (<20.0 ng/mL)	6 (5.08)	73 (43.45)	<0.001
Insufficient (<30.0 ng/mL ≥20 ng/ML)	59 (50.00)	57 (33.93)	
Sufficient (≥30 ng/mL)	53 (44.92)	38 (22.62)	
Platelets ($\times 10^3 cells/mm^3$)†	226.14 (±5.95)	225.8 (±7.39)	0.303
Leukocyte ($cells/mm^3$)	6700 (±221)	9529 (±250)	<0.001
ESR (mm/h)†	11.80 (1.00)	20.05 (±1.70)	0.002
hsCRP (mg/L)†	2.77 (±0.26)	19.93 (±2.29)	<0.001
Ferritin (ng/mL)†	153.98 (±12.38)	273.74 (±27.35)	0.002
TNF-α (pg/mL)†	4.61 (±1.02)	6.50 (±1.06)	0.014
IL-6 (pg/mL)†	13.25 (±39.89)	21.05 (±2.53)	<0.001
IL-10 (pg/mL)†	7.54 (±0.51)	11.85 (±2.63)	0.088

The continuous variables were expressed as mean ± standard error of mean (SEM); the categorical variables were expressed as number (n) and percentage (%)

BMI body mass index, *ESR* Erythrocyte Sedimentation Rate, *hsCRP* high sensitivity C reactive protein, *TNF-α* tumor necrosis factor alpha, *IL* interleukin

† These variables are processed in Ln transformation

Regarding the inflammatory markers, ESR levels were higher in patients with VDD than those with VDS ($p = 0.016$) and hsCRP and serum IL-6 levels were higher in patients with VDD than in patients with VDI and VDS ($p = 0.002$ and $p = 0.001$, respectively) (Table 4). After multinomial logistic regression analysis with these subgroups of patients as dependent variable and those variables with p-value <0.10 described in Tables 3 and 4 as explanatory variables, VDD status remained significantly associated with sex and serum hsCRP levels of the patients ($p = 0.023$ and $p = 0.043$, respectively). In addition, these variables remained significant after Bonferroni post-test (Table 5).

When we analyzed the association between outcome of the patients after three-months follow-up and serum 25(OH)D levels, those with poor outcome had lower 25(OH)D levels than those with good outcome ($p = 0.010$) (Fig. 1a). Moreover, serum 25(OH)D levels showed negative correlation with mRS at three-months follow-up ($r = -0.250$, $p = 0.005$) (Fig. 1b). After logistic regression analysis adjusted for age, sex and mRS baseline, serum 25(OH)D levels remained significantly associated with poor short-term outcome ($p = 0.008$) (Table 6).

Discussion

The main finding of the present study is that VDD can be considered an independent marker associated with acute ischemic stroke. Even after controlling for the classical variables associated with the occurrence of this ischemic event, such as age, sex, ethnicity, BMI, smoking status, presence of DM, dyslipidemia and medications used prior to the ischemic event (antihypertensive, hypoglycemic and hypolipemiant

Table 2 Odds ratio (95% confidence interval) of acute ischemic stroke for vitamin D status at baseline

Vitamin D status	Model 1	Model 2	Model 3	Model 4	Model 5
Deficient	16.32	16.07	14.60	14.44	16.64
(<20.0 ng/mL)	(5.89–45.17)	(5.77–44.70)	(5.21–40.53)	(5.15–40.53)	(5.66–42.92)
	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Insufficient	1.615	1.44	1.40	1.39	1.27
(<30.0–≥20 ng/mL)	(0.87–2.98)	(0.77–2.72)	(0.74–2.65)	(0.73–2.64)	(0.63–2.54)
	$p = 0.127$	$p = 0.250$	$p = 0.291$	$p = 0.308$	$p = 0.39$
Sufficient	1	1	1	1	1
(≥30 ng/mL)	(Reference)	(Reference)	(Reference)	(Reference)	(Reference)

CI: confidence interval

Model 1: adjusted for age (years), gender, body mass index levels (kg/m^2), and ethnicity

Model 2: additionally adjusted for smoking status (yes/no)

Model 3: additionally adjusted for diabetes (yes/no)

Model 4: additionally adjusted for dyslipidemia (yes/no)

Model 5: additionally adjusted for antihypertension, hypoglycemic, and hypolipemiant drugs

Table 3 Demographic, epidemiological, and clinical characteristics of acute ischemic stroke patients, according to their vitamin D status

Variables	Ischemic Stroke Patients			*p*-value
	Vitamin D Deficient [A] ($n = 73$)	Vitamin D Insufficient [B] ($n = 57$)	Vitamin D Sufficient [C] ($n = 38$)	
Vitamin D (ng/mL)[†]	13.85 (±0.51)[B,C]	23.83 (±0.36) [A,C]	37.30 (±1.48) [A, B]	<0.001
Age (years)	71.27 (±1.50) [B,C]	66.47 (±1.60) [A]	63.34 (±2.40) [A]	0.008
Sex				
Male	32 (33.33)	35 (36.46)	29 (30.21)	0.003
Female	41 (56.94) [B,C]	22 (30.56) [A]	9 (12.50) [A]	
Ethnicity				
Caucasian	58 (43.94)	44 (33.33)	30 (22.73)	0.951
Non Caucasian	15 (41.67)	13 (36.11)	8 (22.22)	
BMI (kg/m^2)	26.76 (±0.94)	26.32 (±0.76)	26.07 (±0.79)	0.850
Diabetes mellitus	32 (43.83)	21 (36.84)	11 (28.95)	0.300
Dyslipidemia	33 (45.20)	30 (52.63)	11 (28.95)	0.090
Smoking	14 (19.18)	15 (26.31)	8 (21.05)	0.745
Stroke subtypes				
LAAS	21 (28.78)	25 (43.87)	12 (31.58)	0.861
LAC	26 (35.61)	15 (26.31)	12 (31.58)	
CEI	14 (19.18)	7 (12.28)	5 (13.15)	
ODE	2 (2.74)	2 (3.51)	1 (2.63)	
UDE	10 (13.69)	8 (14.03)	8 (21.06)	
mRs at baseline[a]	4.00 (4.00–5.00)[B,C]	4.00 (3.00–4.50)[A]	4.00 (2.00–4.00)[A]	0.047
mRS after three-months follow-up[b]	5.00 (3.00–6.00)	4.00 (1.00–6.00)	3.00 (1.00–6.00)	0.068

The continuous variables were expressed as mean ± standard error of mean (SEM)

[a] Analyzed 157 patients; [b] Analyzed 134 patients. mRS values were expressed as median and interquartile range (25–75%)

LAAS Large Artery Atherosclerosis Stroke, *LAC* Lacunar infarct, *CEI* Cardio-Embolic Infarct, *ODE* Other determined etiology, *UDE* Undetermined etiology, *mRS* modified Rankin Scale

Table 4 Inflammatory markers of acute ischemic stroke patients, according to their vitamin D status

Variables	Ischemic Stroke Patients			*p*-value
	Vitamin D Deficient [A] ($n = 73$)	Vitamin D Insufficient [B] ($n = 57$)	Vitamin D Sufficient [C] ($n = 38$)	
Platelets ($\times 10^3$cells/mm^3)	224.42 (±9.67)	236.11 (±16.8)	225.83 (±9.04)	0.510
Leukocyte (cells/mm^3)	9464 (±423)	9547 (±371)	9629 (±517)	0.967
ESR (mm/h)[†,]	23.47 (±2.90) [C]	19.77 (±2.70)	14.16 (±2.50) [A]	0.016
hsCRP (mg/L)[†]	30.09 (±4.57) [B,C]	14.40 (±2.39) [A]	8.55 (±2.02) [A]	0.002
Ferritin (ng/mL)[†]	286.30 (±41.54)	252.44 (±44.18)	281.64 (±64.33)	0.877
TNF-α (pg/mL)[†]	4.13 (±0.52)	9.83 (±1.83)	6.55 (±2.67)	0.101
IL-6 (pg/mL)[†]	28.92 (±4.93)[B,C]	16.07 (±3.28)[A]	11.46 (±2.42) [A]	0.001
IL-10 (pg/mL)[†]	13.72 (±5.22)	9.10 (±1.83)	12.35 (5.19)	0.357

The continuous variables were expressed as mean ± standard error of mean (SEM)

[†] These variables are processed in Ln transformation

ESR Erythrocyte Sedimentation Rate, *hsCRP* high sensitivity C reactive protein, *TNF-α* tumor necrosis factor alpha, *IL* interleukin

drugs), patients with VDD were 16.64 more likely to have acute ischemic stroke when compared to those with VDS status. In addition, VDD status was independently associated with higher levels of hsCRP and short-term outcome in patients with acute ischemic stroke.

The association between low levels of 25(OH)D and ischemic stroke is consistent with previous studies performed in other populations worldwide (Pilz et al. 2008; Sun et al. 2012; Brøndum-Jacobsen et al. 2013; Chaudhuri et al. 2014). Studies in both animals and humans demonstrated that vitamin D decreases renin-angiotensin-aldosterone system activity, regulating the genes involved in renin production (Li 2003). VDD has been associated with increased prevalence of hypertension and endothelial dysfunction in healthy subjects and the replacement of vitamin D has shown favorable effects on endothelial function (Tarcin et al. 2009). Moreover, vitamin D produced a significant reduction in the sera lipid profile, CRP, and adhesion molecules in an experimental model (Malek and Shata 2014), suggesting beneficial effects on inflammation and the atherosclerotic process. However, other studies have not found association between VDD and stroke (Bolland et al. 2010; Drechsler et al. 2010; Kühn et al. 2013; Gupta et al. 2014; Majumdar et al. 2015).

The 25(OH)D is widely considered a superior biomarker when compared to 1,25$(OH)_2$D for assessing vitamin D status, because its longer half-life (15 days *vs* 15 h) (Jones 2008). Only two studies measured 1,25$(OH)_2$D, and low levels of this active form were associated with high risk for stroke after 10 years follow-up (Marniemi et al. 2005), as well as it was also considered an independent predictor for fatal stroke (Pilz et al. 2008).

The association between VDD and high levels of hsCRP in patients with ischemic stroke obtained in the present study can be explained by the anti-inflammatory activities of vitamin D, including the inhibition of IL-6 synthesis by monocytes, which is the primary stimulus for CRP production in the liver (Zhang et al. 2012). The first cells responding to brain ischemia are glial cells, particularly microglia, with transcription of early pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, which are able to activate additional inflammatory pathways leading to the induction of positive acute phase proteins, including CRP. Only two studies analyzed the relationship between vitamin D and inflammatory markers in patients with ischemic stroke. Similarly to the results obtained in the present study, Tu et al. (2014) showed that levels of 25(OH)D were inversely correlated with CRP (Tu et al. 2014), whereas, Park et al. (2015) did not find this association in patients with acute ischemic stroke (Park et al. 2015); these conflicting results can be partially explained by the inclusion of patients who were allowed entering the study up to 7 days after the ischemic event.

Increased serum levels of hsCRP have been considered a marker for atherothrombotic disease and may reflect the level of inflammatory activity in atherosclerotic plaques (Shrivastava et al. 2014) as well as it is a sensitive indicator of initial and recurrent ischemic stroke (Di Napoli et al. 2001; Arenillas et al. 2003). CRP acts on endothelial cells inducing tissue factor expression and promotes smooth muscle and endothelial cell proliferation (Cirillo et al. 2005). Moreover, CRP is a chemoattractant for monocytes, up regulates adhesion molecules (Pasceri et al. 2000), modulates nitric oxide bioavailability (Venugopal et al. 2002), increases plasminogen activator inhibitor–1 expression (Chen et al. 2008), and induces several inflammatory genes via NF-κB activation in endothelial human cells (Hattori et al. 2003). Therefore, CRP is directly associated with a proatherothrombotic phenotype in the vessel wall.

Table 5 Results of multinomial logistic regression analysis between explanatory variables and vitamin D status of acute ischemic stroke patients

Variables	Wald for contrasts	*p*-value	Odds ratio	95% CI
Age (Years)	A vs B: 2.186	0.139	0.971	0.934–1.010
	A vs C: 1.905	0.168	1.032	0.987–1.079
	B vs C: 0.071	0.933	1.002	0.960–1.045
Sex (female)	A vs B: 2.154	0.142	0.483	0.183–1.276
	A vs C: 5.169	0.023	3.989	1.210–13.146
	B vs C: 1.163	0.281	1.927	0.585–6.347
Dyslipidemia	A vs B: 0.001	0.992	0.995	0.380–2.609
	A vs C: 3.430	0.064	3.114	0.937–10.347
	B vs C: 3.729	0.053	3.098	0.983–9.765
hsCRP (mg/L)	A vs B: 2.895	0.089	0.980	0.957–1.003
	A vs C: 4.076	0.043	1.038	1.001–1.077
	B vs C: 0.762	0.383	1.017	0.979–1.057
IL-6 (pg/mL)	A vs B: 0.453	0.501	0.994	0.978–1.011
	A vs C: 0.651	0.420	1.009	0.987–1.033
	B vs C: 0.087	0.769	1.004	0.979–1.029
ESR (mm/h)	A vs B: 0.663	0.416	0.991	0.969–1.013
	A vs C: 0.811	0.368	1.014	0.983–1.047
	B vs C: 0.90	0.764	1.005	0.973–1.038
mRs at baseline	A vs B: 0.058	0.809	0.944	0.591–1.507
	A vs C: 0.193	0.666	0.885	0.512–1.529
	B vs C: 0.497	0.481	0.835	0.506–1.378
mRS after three-months follow-up	A vs B: 0.135	0.519	0.917	0.704–1.194
	A vs C: 1.026	0.311	1.167	0.866–1.572
	B vs C: 0.217	0.641	1.070	0.806–1.402

CI confidence interval, *ESR* Erythrocyte Sedimentation Rate, *hsCRP* high sensitivity C reactive protein, *TNF-α* tumor necrosis factor alpha, *IL* interleukin, *mRS* modified Rankin Scale

[a] Vitamin D deficient, [b] Vitamin D insufficient, [c] Vitamin D sufficient

Prospective studies with healthy individuals have proposed that, regardless of other cardiovascular risk factors, increased plasma hsCRP levels are significant predictors of ischemic stroke and transient ischemic attack (TIA) (Rost et al. 2001). Moreover, acutely elevated CRP showed significant and positive association with unfavorable short-term outcome (Muir et al. 1999; Di Napoli et al. 2001; Winbeck et al. 2002).

In the present study, we demonstrated an association between low levels of 25(OH)D and poor outcome after three-months follow-up, independently of age, sex, and the neurological deficit at the admission of the patients. This result suggests that hypovitaminosis D could exacerbate stroke injury affecting post-stroke inflammatory responses, which play a critical role in the pathophysiology of cerebral ischemia (Vila et al. 2000).

It is likely that vitamin D functions to dampen cell-mediated immune responses preventing the strong Th1 responses through acting on the antigen presenting cells, while simultaneously acting on the T cell to enhance Th2 cell development (Boonstra et al. 2001). In addition to its ability to induce a switch from Th1 to Th2 response, vitamin D increases the anti-inflammatory Treg development and cytokine production, whereas Th17 subset is suppressed (Tang et al. 2009). All these effects suggest a possible mechanism by which VDD may exacerbate the cerebral inflammation and neuronal ischemic cell death. The possible modulation of the inflammatory response after stroke by anti-inflammatory mechanisms, including vitamin D, is important due to the direct association between inflammation and secondary damage following stroke.

Moreover, vitamin D induces neuroprotection by increasing neurotrophic factors, such as nerve growth factor (NGF), neurotrophins, and glial cell line-derived neurotrophic factor (GDNF) (Neveu et al. 1994; Wang et al. 2000; Balden et al. 2012). Therefore, VDD may also exacerbate ischemic neuronal cell loss by decreasing the availability of neuroprotective growth factors.

Our results are also consistent with those that showed an association between hypovitaminosis D and poor outcome and mortality in stroke (Kühn et al. 2013; Brøndum-Jacobsen et al. 2013; Daubail et al. 2013, 2014; Wang et al. 2014; Park et al. 2015). Of note, Durup et al.

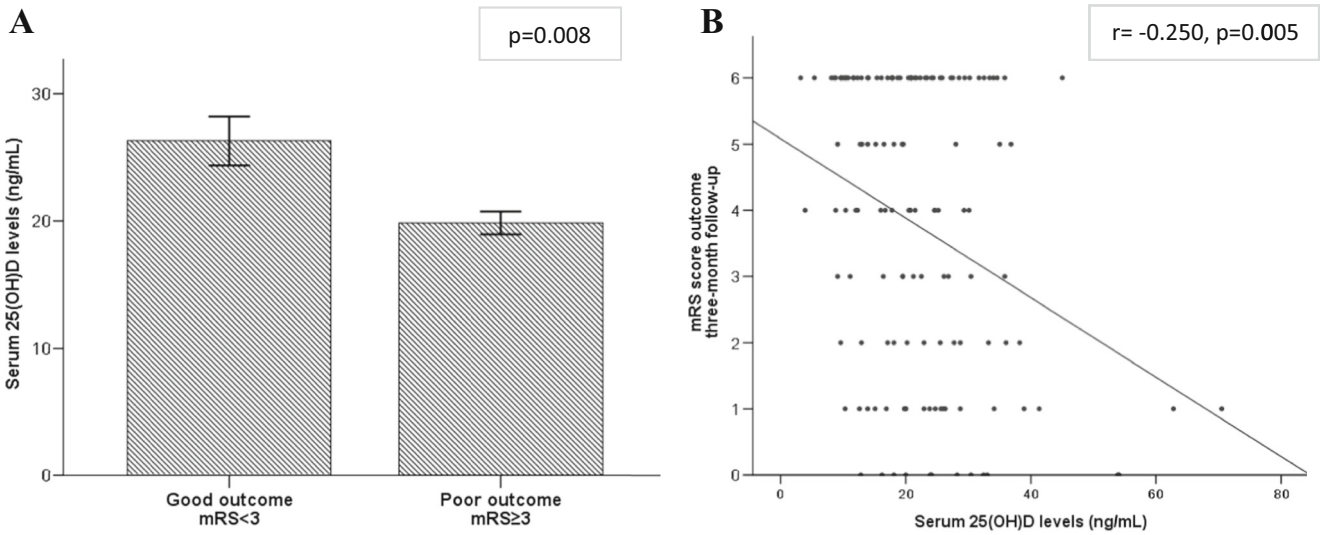

Fig. 1 Serum 25-hydroxyvitamin D [25(OH)D] levels according to the short-term outcome of patients with acute ischemic stroke. **a** Association between serum 25(OH)D levels and short-term outcome evaluated using modified Rankin Scale (mRS) three-months follow-up. Good outcome (mRS < 3) and poor outcome (mRS ≥ 3); the analyze was adjusted for age, sex and mRS baseline, $p = 0.010$. **b** Pearson correlation between serum 25(OH)D levels and functional impairment (mRS) after three-months follow-up ($r = -0.250$, $p = 0.005$)

(2015) showed a nonlinear association between 25(OH)D and stroke mortality with both low and high levels of 25(OH)D. These results should be taken into account regarding the importance to define the appropriate levels of vitamin D that may be sufficient to its major biological functions. In contrast, Ford et al. (2014) showed that although vitamin D supplementation might protect against cardiac failure in older people, it does not offer protection against myocardial infarction or stroke.

Some limitations deserve to be discussed, such as the unavailable data on potential confounding factors, including serum parathyroid hormone, calcium concentrations, data on sun exposure, dietary intake of vitamin D and outdoor physical activity. Moreover, the level of hsCRP was measured only once for each patient and could not be able to reflect its potentially dynamic changes in ischemic stroke and the samples were not obtained just after the ischemic stroke symptoms onset, but as soon as possible after admission of the patients to hospital. However, previous studies suggested that the level of hsCRP in ischemic stroke patients varies 7 days after symptom onset (Worthmann et al. 2010) and that the high level of CRP obtained within 30 days of stroke had a strong predictability for long-term survival of patients study (Whiteley et al. 2011). On the other hand, the strength of this study is that we have found an association between low vitamin D status with acute ischemic stroke, hsCRP, as well as with short-term outcome, which remained significant after adjusting for several confounder variables that could have influenced the results.

Table 6 Binary logistic regression analyses with acute ischemic stroke short-term outcome as dependent variable and explanatory variables

Variables	Wald	*p*-value	Odds ratio	95% CI
Age (years)	0.779	0.377	1.016	0.981–1.053
Sex (female)	2.962	0.085	.407	0.146–1.33
Vitamin D (ng/mL)	7.085	0.008	.929	0.880–0.931
mRS at baseline	25.663	<0.001	3.208	2.044–5.037

CI confidence interval, *mRS* modified Rankin Scale

Conclusion

Although the current study design does not allow to link the VDD with the causality of acute ischemic stroke, the independent association observed between vitamin D status with hsCRP, that is considered a valuable and sensitive indicator of initial and recurrent cerebrovascular events, as well as with functional impairment at short-term outcome, underscores the important role of vitamin D in the ischemic stroke pathophysiology. Further studies with large sample size of individuals are warranted to better understand the anti-inflammatory mechanisms of vitamin D and its involvement in acute ischemic stroke.

Compliance with ethical standards

Conflict of interest All the authors declare that there is no conflict of interest.

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