



Association of Serum 25-hydroxyvitamin D in Multiple Sclerosis: A Study from South India

Jaydip Ray Chaudhuri¹, Kandadai Rukmini Mridula², Matapathi Umamahesh³, Banda Balaraju⁴ and VCS Srinivasara Bandaru^{1,5*}

¹Department of Neurology, Yashoda Hospital, India

²Department of Neurology, Nizam's Institution of Medical Sciences, India

³Department of Radiology, Yashoda Hospital, India

⁴Department of Medicine, Yashoda Hospital, India

⁵Department of Clinical Research, Yashoda Hospital, India

Abstract

Studies have incriminated vitamin D deficiency to be associated with Multiple Sclerosis (MS). To investigate association between serum 25-hydroxyvitamin D and MS.

Methods: We recruited 56 MS patients and 56 age and sex matched controls from Yashoda hospital, study period from August 2011 to July 2016. All cases and controls were evaluated for various risk factors and underwent tests for serum 25-hydroxyvitamin D, lipid profile estimation. Expanded Disability Status Scale (EDSS) was assessed in cases and dichotomized to low (≤ 3) and high (≥ 3.5) impairment.

Results: Out of 56 patients, men were 28 (50%), serum 25-hydroxyvitamin D deficiency was significantly higher in cases 39 (76.4%) compared to controls 14 (27.4%) ($p < 0.0001$). Mean total cholesterol, mean triglycerides ($p < 0.0001$) and mean Low-Density Lipoprotein (LDL) ($p = 0.03$) levels were significantly higher, while mean High-Density Lipoprotein (HDL) levels were significantly lower in cases. Among MS subtypes, 25-hydroxyvitamin D deficiency found 33 (84.6%) in Relapsing-Remitting MS (RRMS), 3 (7.6%) in Secondary-Progressive MS (SPMS) and 3 (7.6%) in Clinically Isolated Syndrome (CIS).

Elevated mean total cholesterol, LDL, mean triglycerides levels, low mean HDL and high EDSS were significantly associated with 25-hydroxyvitamin D deficiency in cases ($p = 0.01$). After adjustment using multiple logistic regression analysis, serum 25-hydroxyvitamin D deficiency an independent association with MS (Odds: 2.5; 95% CI: 1.1-5.2) and high EDSS (Odds: 3.5; 95% CI: 2.1-18.4).

Conclusion: In our study, we found serum 25-hydroxyvitamin D deficiency an independently associated with MS and high EDSS.

Keywords: Serum 25-hydroxyvitamin D; MS subtypes; RRMS; SPMS; CIS; EDSS; Dyslipidemia; Indian study

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease, involving the central nervous system, associated with axonal loss, demyelination and brain atrophy. MS affects both genders but predominate in females [1]. MS can occur in all age groups, with middle aged people being most involved [2]. There is a strong belief based on several studies, that environmental factors such as viral infections, high ultraviolet radiations, fish and animal fat intake, dairy product consumption [3], vitamin D levels [4] and temperate climate [5] can affect the occurrence of MS Worldwide 2.5 million people are afflicted, every week 200 new cases occur though there is lower prevalence in Africans, Native Americans, and Asians [6]. In India, the prevalence rate in a study was 8/100,000 [7]. Recent studies have suggested a strong association of vitamin D deficiency with MS [7,8]. Vitamin D is a 9,10-seco steroid which exists in many forms in the environment and humans including vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol). Vitamin D is necessary for the human body to maintain bone metabolism and stabilize the balance between calcium and phosphorus [9]. But its role extends beyond this and its role in maintaining our immune system and inflammation is being unraveled slowly. Serum 25-hydroxyvitamin D is a marker of vitamin

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*Correspondence:

VCS Srinivasara Bandaru, Department of Neurology and Clinical Research, Yashoda Hospital, Hyderabad, 500082 India, Tel: 9441266519; Fax 9104023398667;

E-mail: rsbhandaru@gmail.com

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Table 1: Baseline characteristics.

Parameters	Cases (n=56)(%)	Controls (n=56) (%)	p value
Men	28(50%)	28(50%)	0.8
Mean age	40.2 ± 13.5	42.3 ± 10.1	0.6
Age range	18-69	18-69	
Mean levels 25-hydroxyvitamin D (ng/mg)	14.8 ± 6.1	24.5 ± 5.9	<0.0001
Deficiency of 25-hydroxyvitamin D	39(69.6%)	14(25%)	<0.0001
Hypertension	8(14.2%)	12(21.4%)	0.3
Diabetes	7(12.5%)	5(8.9%)	0.4
Smoking	9(16%)	8(14.2%)	0.9
Alcoholism	5(8.9%)	6(10.7%)	0.9
Obesity	8(14.2%)	5(8.9%)	0.9
Dyslipidemia	28(53.5%)	18(32.1%)	0.05
Mean total cholesterol (mg/dL)	201.9 ± 19.1	173.1 ± 18.1	<0.0001
Mean HDL(mg/dL)	38.6 ± 2.1	41.9 ± 2.6	<0.0001
Mean LDL(mg/dL)	144.6 ± 18.7	137.4 ± 16.1	0.03
Mean VLDL(mg/dL)	24.2 ± 2.8	25.1 ± 2.1	0.6
Mean triglycerides(mg/dL)	144.7 ± 8.7	136.5 ± 7.9	<0.0001
Mean alkaline phosphatase (μ/L)	124.2 ± 34.1	84.1 ± 25.4	<0.0001
Mean serum phosphorous (mg/dL)	3.4 ± 1.2	4.8 ± 0.7	<0.0001
Season of presentation			
Summer	27(48.3%)	29(51.7%)	0.5
Winter	29(51.7%)	27(48.2%)	0.5

D status in the human body [9]. The aim of study, to investigate the association between serum 25-hydroxyvitamin D levels and MS. Very limited studies are available on this topic from the South India.

Material and Methods

We consecutively recruited 56 patients with MS from the Department of Neurology at Yashoda hospital, which is a major referral center in South India. Fifty six age and sex matched controls were recruited from healthy volunteers. The study period was from August 2011 to July 2016. This study was approved by the Yashoda Institutional Ethics Committee and informed consent was obtained from case and controls.

Inclusion criteria for case controls

All patients (cases), who fulfilled McDonald's criteria (2010) for MS [10] were included as cases. Diagnosis was confirmed by two senior neurologists. The diagnosis was based on clinical history, examination, including evolution of cerebrospinal fluid (elevated Immunoglobulin G [IgG] index or 2 or more oligoclonal bands), NeuroMyelitis Optica (NMO) and Magnetic Resonance Imaging of brain and spinal cord, Evoked potentials (Visual (VEP) and Auditory (BAEP)). We recruited all subtypes of MS, Relapsing-Remitting (RRMS), Secondary-Progressive (SPMS), Primary-Progressive (PPMS), Progressive-Relapsing (PRMS) and Clinically Isolated Syndrome (CIS). Controls were selected from healthy volunteers from the same hospital and did not have any history of neurological illness or visual problems.

Exclusion criteria for case controls

Cases who did not met the McDonald's 2010 criteria, cases and controls who had history of cardiac disease, vasculitis, recurrent stroke,

already on or have received vitamin D supplements, endocrinological dysfunction, substantial abnormalities in hematologic, hepatic, renal or metabolic functions, any condition predisposing to hypercalcemia, nephrolithiasis were excluded.

Assessment of expanded disability status scale (EDSS)

Expanded Disability Status Scale (EDSS) was assessed by in all patients by a single neurologist with expertise in neuroimmune disorders on the day of admission. In the present study we dichotomized those with low EDSS score (≤ 3) and those with high EDSS score (≥ 3.5).

Risk factor evaluation

Demographic data (age, sex), level of education, socioeconomic status, past medical history and physical and neurologic examination, were evaluated by a neurologist. Definitions for hypertension, diabetes, dyslipidemia, smoking, alcoholism, Body Mass Index (BMI) were mentioned in our previous paper [9].

Blood collection

Blood collection was done at the time of enrollment of cases and controls; 5 ml blood sample was used for estimation of 25-hydroxyvitamin D. We used Chemiluminescent Micro Particle Immunoassay (CMIA) with automated instruments for estimation of 25-hydroxyvitamin D levels. Values ≤ 20 ng/ml were diagnosed as 25-hydroxyvitamin D deficiency and values > 20.1 ng/ml as normal [9].

Statistical analysis

Statistical analysis was performed using SPSS 14.0 software (statistical package for the Social sciences, SPSS Inc). Mean+SD (Standard Deviation) was calculated. The paired 't' test was applied to test the differences in continuous variables and Wilcoxon ranked sum test was applied in non parametric data (comparison between controls and MS subtypes). We estimated Odds Ratio (OR) and the resulting 95% CI for the matched case-control pairs. Multiple logistic regressions were performed before and after adjustment for potential confounders (gender, hypertension, diabetes, smoking, alcoholism, dyslipidemia, obesity, serum 25-hydroxyvitamin D and EDSS). All tests were two sided and p value < 0.05 was considered statistically significant.

Results

In this present study, men constituted 50% in cases as well as controls, mean age was 40.2 ± 13.5 years in cases and 42.3 ± 10.1 in controls. Serum 25-hydroxyvitamin D deficiency was found among 39 (69.6%) cases and 14(25%) controls. On comparison of various risk factors, mean total cholesterol was significantly higher in cases (201.9 ± 19.1 mg/dl) compared to controls (173.1 ± 18.1 mg/dl). Mean HDL was 38.6 ± 2.1 mg/dl in cases and 41.9 ± 2.6 in controls. Mean triglycerides levels were 144.7 ± 8.7 in cases and 136.5 ± 7.9 in controls (Table 1).

Out of 56 cases, RRMS were 42 (75%) followed by SPMS 5(8.9%) and CIS 9(16%). When compared to controls, 25-hydroxyvitamin D levels in RRMS (mean 14.3 ± 5.9 ng/ml) and SPMS (14.6 ± 6.5 ng/ml) were significantly lower while CIS had lower levels (17.0 ± 6.7 ng/ml), but didn't reach statistical significance (Figure 1).

Among cases, 25-hydroxyvitamin D deficiency was significantly higher in women (53.8%), dyslipidemia (including elevated total cholesterol, LDL and triglycerides, reduced HDL) and severe

Table 2: Comparison between 25-hydroxyvitamin D deficiency and normal levels in cases.

Parameters	<20(ng/mg) (n=39)	>20(ng/mg) (n=17)	p value
Women	21(53.8%)	3(17.6%)	0.026
Mean age(years)	39.5 ± 12.9	42.5 ± 15.5	0.5
Age range (years)	19-67	22-69	
Hypertension	5(12.8%)	3(17.6%)	0.6
Diabetes	5(12.8%)	2(11.7%)	0.8
Smoking	5(12.8%)	4(23.5%)	0.2
Alcoholics	3(7.6%)	2(11.7%)	0.5
Obesity	7(13.7%)	1(5.8%)	0.9
Dyslipidemia	24(61.5%)	4(23.5%)	<0.0001
Mean total cholesterol(mg/dL)	207.9 ± 32.1	183.1 ± 22.8	0.01
Mean HDL(mg/dL)	39.6 ± 3.1	42.9 ± 3.5	0.002
Mean LDL(mg/dL)	151.9 ± 38.6	126.7 ± 36.5	0.05
Mean VLDL(mg/dL)	25.1 ± 3.9	23.4 ± 4.1	0.1
Mean triglycerides (mg/dL)	161.4 ± 12.7	135.1 ± 10.8	<0.0001
MS subtypes			
Relapsing-remitting MS (RRMS)	33(84.6%)	9(52.8%)	0.02
Secondary-progressive MS(SPMS)	3(7.6%)	2(11.7%)	0.9
Clinically Isolated Syndrome (CIS)	3(7.6%)	6(35.2%)	0.008
Expanded Disability Status Scale (EDSS)			
High EDSS (≥ 3.5)	30(76.9%)	6(35.2%)	<0.0001
Low EDSS (≤ 3)	9(23%)	11(64.8%)	<0.0001
Seasonal variation			
Summer	19(48.7%)	8(47%)	0.1
Winter	20(51.2%)	9(53%)	0.4

disease (EDSS score > 3.5) (76.9%), compared to >20(ng/mg) 25-hydroxyvitamin D with MS Significantly higher proportion of RRMS patients i.e., 84.6% had Vitamin D deficiency whereas CIS patients had higher prevalence of normal vitamin D levels (35.2%) (Table 2). After multivariate analysis we established that Serum 25-hydroxyvitamin D (odds 2.5; 95% CI: 1.1-5.2) and EDSS (odds 3.5; 95% CI: 2.1-18.4) were independently associated with MS (Table 3).

Discussion

In our study, we established a significant association of serum 25-hydroxyvitamin D deficiency with MS, these findings were advocated by others [11,12]. The present study noted three fold higher prevalence of 25-hydroxyvitamin D deficiency among MS patients 39 (76.4%) compared to controls 14 (27.4%). Our findings were supported by others [13-16]. Pandit et al. [13] noted in her study that 71% of MS patients and 53.7% among controls presented with 25-hydroxyvitamin D deficiency. A recent study by Ibrahim et al had similar findings, 65 % of MS patients had low levels of 25-hydroxyvitamin D compared to 20% among controls [14]. Nieves et al. [15] demonstrated 25-hydroxyvitamin D insufficiency in 77% MS patients, while Fjeldstad et al. [16] noted 25-hydroxyvitamin D deficiency in 66%.

Our study found a significantly lower mean level of 25-hydroxyvitamin D (14.8 ± 6.1 ng/mg) in MS patients compared to 24.5 ± 5.9 ng/mg in controls. Our findings were supported by

Table 3: Multiple logistic regression before and after adjustment.

Parameters	Before adjustment		After adjustment	
	Odds	95%CI	Odds	95%CI
Serum 25-hydroxyvitamin D	5.8	2.9-15.7	2.5	1.1-5.2
Expanded disability status scale	12.0	4.7-40.2	3.5	2.1-18.4
Dyslipidemia	2.1	0.98-4.5	*	*

* Number insufficient.

Fjeldstad et al. [16], who noted a mean of 17.7 ng/mg among MS patients, while in the study by Pandit et al. [13] values of 39.0 nmol/l in patients and 46.5 nmol/l in controls were noted.

Several studies have established a possible association of insufficient levels of 25-hydroxyvitamin D with the incidence of MS [17,18]. Eskandari et al. [8] suggested vitamin D deficiency as one of underlying mechanism of increased MS incidence.

Recent studies have reported an association of higher vitamin D levels with lower relapse rates in MS patients. Concomitant serum 25(OH) vitamin D levels have been found to be lower during relapses when compared to remissions in MS patients [11,19,20].

Runia et al. [18] showed an odds ratio for monthly exacerbation rates of 0.15 for vitamin D levels below 50 nmol/l, 0.10 for 50 nmol/l to 100 nmol/l and 0.07 for more than 100 nmol/l and he suggested that vitamin D supplementation decreased exacerbation risk in RRMS.

Various subtypes of MS have shown different prevalence of 25-hydroxyvitamin D deficiency. The present study noted a highest prevalence among RRMS 33(84.6%) followed by SPMS 3(7.6%) and lowest among CIS 3(7.6%), these finding advocated by others [18]. A recent study showed 83% of patients had insufficient and 17% deficiency of vitamin D in RRMS [21]. Our study noted a mean value of 25-hydroxyvitamin D 14.3 ng/ml in RRMS patients. A similar finding was noted by Thouvenot et al. [22] he noted in his study a mean value of 22.1 ng/ml in RRMS. In our study the mean values of serum 25-hydroxyvitamin D in SPMS (14.6 ng/ml) and CIS (17 ng/ml) were higher. Thouvenot et al [22,23] had lower levels of Vitamin D in SPMS (15.6 ± 14.3) and primary progressive multiple sclerosis (PPMS) (15.8 ± 8.9). We did not have any PPMS patients in our study.

A protective role of vitamin D on MS disease course is biologically plausible. Low levels of vitamin D during acute exacerbations in comparison to remissions have been documented [20]. Vitamin D deficiency is one of the environmental factors associated with the development of MS [24].

Various experimental settings have implied it. Vitamin D receptors an especially for 1.25 dihydroxy vitamin D are present on various cells of the immune system like macrophages and activated T and B cells [23]. The stimulation of these receptors in vitro causes inhibition of inflammatory cytokines production [25]. In a recent study in MS patients, vitamin D supplementation reduces IL-2 mRNA levels in peripheral blood mononuclear cells [26].

They also promote development of regulatory T cells and correlation also has been demonstrated between serum 25-hydroxyvitamin D levels and a more anti inflammatory ratio of T helper cells type 1 and type 2 (Th1/Th2) [27]. Apart from MS a more diffuse protective role of Vitamin D against a variety of inflammatory diseases such as rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus is becoming evident [28]. Prospective studies however are scarce.

In MS, the immunomodulation by 1,25-hydroxyvitamin D, can reduce severity by inhibiting proinflammatory cells and increasing anti-inflammatory cells. Further modification in cytokine profile may reduce inflammation and have been demonstrated in MS patients following dietary intake of vitamin D (1000 IU/ day) plus calcium (800 mg/day). However, more studies are essential to identify the exact effect of vitamin D on MS pathology [29].

The following are assessed according to Hill's criteria [30].

1. Strength of association
2. The consistency of association
3. Temporality, i.e., if the exposure occurs prior to the disease
4. Biological gradient or a clear cut correlation between the severity of deficiency and the severity of disease
5. Plausibility of causation
6. Lastly experiments to demonstrate a change in disease frequency with alteration in exposure.

In the present study criteria 1, a moderately strong association was found and the odds of the association of 25-hydroxyvitamin D deficiency with MS was 2.5 and similar finding were found by Banwell et al. [31].

Our findings are supported by previous research and as discussed previously, the causality is biologically plausible (criteria 2 and 5). As the assessments were done cross sectional and hence might not evaluate temporality, there is a possibility of reverse causality and hence only intervention trials in future can help in identifying the relationship between vitamin D supplementation and disease course in MS [18].

In the present study, 76.9% of patients with high EDSS score (≥ 3.5) were associated with deficiency of 25-hydroxyvitamin D. Our study was advocated by others [12]. A statistically significant inverse correlation was found between 25-hydroxyvitamin D level and EDSS score ($p = 0.016$) [11]. Recent studies demonstrated that increasing disability was strongly associated with lower levels of 25-hydroxyvitamin D and patients with higher EDSS were more likely to have vitamin D insufficiency [12,32]. Thouvenot et al. [22] established in his study that vitamin D levels >20 ng/ml were 2.5 times more likely to have an EDSS score below 4.

Our study demonstrated no significant association between 25-hydroxyvitamin D deficiency and low EDSS score (≤ 3). A similarly study showed no significant association was found between EDSS score <4 and low vitamin D levels but had a significant association with an EDSS > 4 (odds ratio 2.56, 95% CI 1.00–6.67, $p=0.05$) [22].

Nieves et al. [17] demonstrated that very low levels of 25-hydroxyvitamin D (< 50 nmol/l) were significantly associated with mean EDSS score of 6.9. In our study we established after Multiple regression analysis, serum 25-hydroxyvitamin D deficiency was independently associated with high EDSS score (Odds: 3.5; 95% CI:2.1-18.4). However some studies have found no significant association between EDSS and 25-hydroxyvitamin D deficiency [29].

Severe MS itself may contribute to Vitamin D deficiency due to reduced sun exposure and reduced food intake. Hence the causality cannot be confirmed, although we found a strong association between low Vitamin D levels and severe MS.

In our study we noted mean total cholesterol, mean HDL, mean triglycerides and mean LDL levels were significantly associated with cases compared to controls, our findings advocated by others [33]. In our study we found VLDL levels were similar in cases and controls. However Omoulu et al. [34] noted in his study VLDL-C levels was significantly higher in progressive MS patients than those of the controls. High levels of lipid profiles have associated with MS and 35% of MS patients had TC > 5.5 mmol/l [35,36]. A recent study showed a beneficial effect of high-dose simvastatin in reducing whole-brain atrophy compared with placebo in MS [37]. Giubilei et al. [36] inferred that plasma cholesterol level may be a potential marker of disease burden in patients with MS at the onset of disease. Dyslipidemia has been associated with vitamin D deficiency. In our study we established elevated mean total cholesterol, mean HDL, mean LDL and mean triglycerides significantly associated with 25-hydroxyvitamin D deficiency in MS patients.

The exact relationship is not clear. Vitamin D is formed from squalene in the skin on exposure to sun. When this reaction is reduced due to various factors like reduced sun exposure or increased melanin, there is an increased conversion of squalene to cholesterol. Further hypovitaminosis D is associated with reduced HDL and hence there is aggravation of dyslipidemia [9].

Hypovitaminosis D is also associated with hypertriglyceridemia, and is explained by various mechanisms including possibly reducing the calcium mediated suppression of hepatic triglyceride synthesis, increasing insulin resistance, elevated parathormone mediated reduction in lipolysis and reduced expression of VLDL cholesterol receptors [9].

Gender

In our study we found a higher prevalence of deficiency of 25-hydroxyvitamin D among women (53.8%) ($p=0.05$) with MS. Similar findings are noted by others [17,38]. Yetley demonstrated lower levels of serum 25-hydroxyvitamin D level in women compared to men [38]. Women are more prone to Vitamin D deficiency; they have reduced intake and less sunlight exposure in many parts of the world. In addition with menopause, increased bone turnover occurs in women causing increased requirement of Vitamin D [9]. This can have a heightened emphasis in diseases like MS which are more prevalent in women. In contrast some studies have found no significant association of gender with serum 25-hydroxyvitamin D deficiency in MS [29].

Seasonal variation with MS

In our study we did not find any seasonal variation in 25-hydroxyvitamin D levels in MS patients. We compared the patients who were assessed during summer (March to June) and winter (November to February). In summer 19(48.7%) patients had 25-hydroxyvitamin D deficiency while in winter 20(51.2%) had 25-hydroxyvitamin D deficiency ($p=0.5$). Our study is advocated by Runia et al, noted in his study, no significant differences among the 4 seasons [18]. However some studies have found significant association of vitamin D deficiency with winter in MS [39].

Therapeutic

Several studies have established that Vitamin D supplement is associated with 40% lower risk of MS [18]. A recent study noted that addition of 20,000 IU of 25-hydroxyvitamin D weekly to the treatment of MS resulted in significantly fewer new MRI lesions [18,22,40]. There was a trend towards significance in reducing disease

severity (lower EDSS score), MRI total disease burden and clinical improvement (timed 10 foot tandem walk score) over one year [40]. Still the evidence for vitamin D supplementation is not robust.

Limitations and Strength of Study

Our study has few limitations, MS is a relatively rare disease and our sample size is small. Vitamin D and lipid profile samples were taken after the diagnosis of MS in the patient population. As it is a cross sectional study, we can only show an association. We have not assessed the effect of sunlight exposure on Vitamin D levels. The strengths of the study was that the samples were all analyzed by a single laboratory reducing inter-rater variability.

Conclusion

This study establishes an independent association of 25-hydroxyvitamin D with MS and disease severity (EDSS \geq 3.5). Dyslipidemia and female gender also significantly association with MS in indian population. The association is similar to rest of the world. As it has been demonstrated recently that a high prevalence of vitamin D deficiency is prevalent in Indians, it may be a significant factor in MS disease management. A future large multi centre study on effect on Vitamin D levels and the role supplementation on MS in Indian population may clarify its role.

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