Association of Vitamin D Metabolite Levels with Relapse Rate and Disability in Multiple Sclerosis

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ABSTRACT:

BACKGROUND:

Multiple sclerosis is an inflammatory demyelinating disease of the central system. It estimated to affect more than 2 • 5 million people worldwide. It is the most common non-traumatic cause of disability in young adults. Although the cause of multiple sclerosis remains undetermined, number of risk factors for MS have been identified and they can loosely be put into one of two categories; genetic or environmental components. Epidemiologic studies have suggested there is an increase in incidence and prevalence of MS with increasing latitude north and south of the equator. Latitude has implicate vitamin D status as a determinant of risk.

OBJECTIVE:

To study the association of vitamin D level with relapse rate and disability in patients with relapsing remitting multiple sclerosis in Iraq.

PATIENTS AND METHODS:

Thirty patients (6 males and 24 females) with relapsing remission multiple sclerosis (RRMS), their age range from 16 to 45 years, recruited from MS clinic of neurology department of Baghdad teaching hospital in the medical city in Baghdad and twenty five completely healthy controls (6 males and 19 females) from general population and their age range from 20 to 40 years were enrolled in this study in the period from April 2011 to the end January 2012.

RESULTS:

The present study shows low vitamin D levels for both patient with RRMS and control group. There is significantly lower 25(OH)D level in patients with relapse compared with patients without relapse in the last 6 weeks. Also we found higher expanded disability status scale (EDSS) in patients with relapse compared with patients without relapse in the last 6 weeks. Lastly, we didn't find any correlation between vitamin D level and EDSS in patients group study.

CONCLUSION:

We concluded from this study that there is low circulating level of 25(OH)D in RRMS patients, especially during relapses. Also there is no effect of vit D on disability

KEY WORDS: relapsing remitting multiple sclerosis , vitamin d level .

INTRODUCTION:

Multiple sclerosis (MS) is an inflammatory disease in which the fatty myelin seaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of clinical signs and symptoms [(1)] . MS is a chronic, progressively disabling disease of the CNS, estimated to affect more than 2.5 million people worldwide [(2,3)]. Vit. D is a steroid hormone with pleiotropic actions on most tissues and cells in the body. Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. The "D" represents D2 or D3 [(4,5)].

In humans, vit.D3 is produced in the skin following exposure to ultra violet B radiation and can also be obtained from the diet [(4,5)]. Dietary intake is unlikely to meet the daily body's needs. Therefore, casual exposure of the skin to sunlight might be the major source of the vitamin for most people [(6)]. Recent studies suggest that vit.D is an important environmental factor [(7)]. In the murine model, EAE , supplementation with both vit.D metabolites [25(OH)D and 1,25(OH)2D] prevents the disease or puts the disease into remission. Raising the circulating levels of 1,25(OH)2D by

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supplementing 1,25(OH)2D effectively prevents or ameliorates EAE [8]

Through a limited number of studies, specific single nucleotide polymorphisms of the VDR have been linked to lower vit.D metabolism in individuals with MS, as well as greater susceptibility to disease and One of the strongest mechanistic links between vit.D and MS comes from a recent study demonstrating that calcitriol modulates the expression of the particular HLA-DRB1 allele most consistently associated with increased risk of MS, HLA-DRB1 *1501. [9-10].

Serum 25 (OH)D levels in adults recently diagnosed with MS are low relative to controls. In a study from Finland, serum 25(OH)D concentrations were significantly lower in adults diagnosed with MS in the period of June through September compared to healthy controls samples in the same time period.[10,11]

Lower 25(OH)D levels have been reported in MS compared with healthy control populations. In MS populations, EDSS scores correlate negatively with circulating levels of 25(OH)D, and these levels are lower during relapses than remission in RRMS [11-12].

Vit.D concentrations also correlate with some types of MRI evidence of MS disease activity. In one study, low serum 25(OH)D levels predicted an increased likelihood of gadolinium-enhancing lesions in MRI scans performed in the subsequent two month period (13-14-15).Although, lower serum 25(OH)D was observed in relapses, serum 25(OH)D did not correlate with MRI burden of disease but, importantly, gadolinium-enhanced images were not included in this study.

PATIENTS AND METHODS :

SUBJECTS:

A case-control study included 30 patients (6 males and 24 females) were diagnosed as MS, their age range from 16 to 45 years and twenty five control group (6 males and 19 females) individuals from general population who were apparently healthy and their age range from 20 to 40 years enrolled in this study. Patients were recruited from the MS clinic of neurology department of Baghdad teaching hospital in medical city, Baghdad, during the period from April 2011 to the end January 2012.

Eligibility and exclusion criteria:

Patients whom started the disease course as a relapsing remitting form with duration less than five years were involved , while patients with other disease course were excluded. All patients were on treatment with Interferon B1b.

The diagnosis in each case was established by clinical history and examination, radiologic finding, and laboratory investigations according to modified McDonald criteria .

Base line data about patients were obtained from the history and clinical examination; these include name, gender, age, duration of disease since first symptoms in years, number of relapses within two years before serum sampling , state at time of study (relapse or not) and EDSS at time of serum sampling. All informations were arranged in a protocol paper and fulfilled for each patient. All investigations were performed after obtaining informed consent from patients.

Blood sample collection and preparation:

- Five millitres (5ml) venous blood were obtained from each subject included in the study and placed in a sterile plain tube, then centrifuged and the serum was separated and stored at 2-8 Ċ *Electrochemiluminescence immunoassay(ECLIA)is used forquantitative determination of 25- hydroxy-vitamin D3 in serum:*Competition principle Total duration of assay is 18 minutes. 1st incubation :25-OH vitamin D3 in the sample (35 ML) competes with biotin labeled vitamin D in the complex contained in R2 (biotin–vitamin D and monoclonal 25-OH vitamin D3-specific ruthenium labeled antibody). The remaining amount of the complex (biotin–vitamin D and monoclonal 25-OH vitamin D3-specific ruthenium labeled antibody) is dependent upon the analyte concentration in the sample. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- At the same time PTH and ALP levels were determined and DEXA scans were send for both patients and controls.

Statistics :

Analysis of data was carried out using the available statistical package of SPSS-18 (Statistical Packages for Social Sciences- version 18 "PASW" Statistics).

Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) was tested using analysis of variance (ANOVA) for more than two groups and using independent student-t-test for difference between two means, while different

percentages (qualitative data) were tested using Pearson Chi-square test (χ^2-test). Statistical significance was considered whenever the P value was less than 0.05.

RESULTS:

The study included 30 patients with clinically definite multiple sclerosis with relapsing remitting course attended MS clinic in Baghdad teaching hospital in medical city complex and 25 healthy individuals from general population served as control

Table 1: Demographic characteristics of patients and control groups.

	Patients		Controls		P value
	No	%	No	%	
Gender Male	6	20.0	6	24.0	0.721
Female	24	80.0	19	76.0	
Age (years) 16—24	5	16.7	9	36.0	0.074
25—29	8	26.7	11	44.0	
30—34	4	13.3	1	4.0	
35—39	6	20.0	3	12.0	
40—45	7	23.3	1	4.0	
Mean±SD(range)	32.17±8.24	16-45	26.16±5.58	20-40	

*Significant using Pearson chi-square test at 0.05 level of significance.

Table 2: Shows number and percentage of patients according to occurrence of relapse in last 6weeks and EDSS.

		Patients	
		No	%
Occurrence of relapses in last 6weeks	Yes	15	50.0
	No	15	50.0
EDSS	1	5	16.7
	2	14	46.7
	3	2	6.7
	4	7	23.3
	5	1	3.3
	6	1	3.3
EDSS Mean±SD	2.77±1.38		

Table 3: Shows vitamin D level ,Other serological parameters and DEXA in study groups.

		Patients		Controls		P value
		No	%	No	%	
Vitamin D (ng/ml)	Low	23	76.7	16	64.0	0.303
	Normal (20-42)	7	23.3	9	36.0	
PTH (pg/ml)	Low	-	-	-	-	-
	Normal (8.7-79.6)	30	100.0	25	100.0	
ALP (IU/L)	Low	-	-	-	-	-
	Normal (30-85)	30	100.0	25	100.0	
DEXA scan	Normal (<-1)	11	36.7	16	64.0	0.037*
	Osteopenia (-1 - -2.5)	14	46.7	9	36.0	
	Osteoporosis (>-2.5)	5	16.7	-	-	

*Significant using Pearson chi-square test at 0.05 level of significance.

Table 3 shows results of vit.D level of study groups, which is low in 23 (76.7%) of the patients and in 16 (64.0%) of controls and normal in 7 (23.3) of the patient and 9 (36.0) of controls. This revealed no significant change between the patient and control groups (P value = non significant).

Other parameters PTH, ALP levels were normal in both patients and controls.

DEXA was normal in 11 of patients (36.7) and in 16 (64.0) of controls, and osteopenic range in 14 of patients (46.7) and in 9 (36.0)of controls, and osteoporotic range in 5 (16.7) and nil in control group (p value = significant), as mentioned in Table 3.

Table 4: Distribution of variable parameters including vitamin D level and in patient group study according to the occurrence of relapses in the last 6 weeks.

		Occurrence of relapses in last 6weeks				P value
		Relapse		No Relapse		
		No	%	No	%	
Age (years)	16—24	2	13.3	3	20.0	0.455
	25—29	3	20.0	5	33.3	
	30—34	1	6.7	3	20.0	
	35—39	4	26.7	2	13.3	
	40—45	5	33.3	2	13.3	
Gender	Male	1	6.7	5	33.3	0.068
	Female	14	93.3	10	66.7	
Vitamin D (ng/ml)	Low	14	93.3	9	60.0	0.031*
	Normal (20-42)	1	6.7	6	40.0	
PTH (pg/ml)	Low	-	-	-	-	-
	Normal (8.7-79.6)	15	100.0	15	100.0	
ALP (IU/L)	Low	-	-	-	-	-
	Normal (30-85)	15	100.0	15	100.0	
DEXA scan	Normal (<-1)	5	33.3	6	40.0	0.750
	Osteopenia (-1 - -2.5)	8	53.3	6	40.0	
	Osteoporosis (>-2.5)	2	13.3	3	20.0	

*Significant using Pearson chi-square test at 0.05 level of significance.

The study revealed the results of vit.D level in RRMS study groups according to state at presentation (with relapse or without relapse in the last 6 weeks), in which the result was low in 14 (93.3%) in the patients with relapses and 9 (60.0%) in the patients without relapse also the results were normal in 1 (6.7%) in the patients with relapses and in 6 (40%) in patients without relapse .This revealed significant change between groups of patient according to state at presentation (with relapse or without relapse) (P value = significant),as mentioned in Table 4 and shown in figure 1.

Other parameters (PTH,ALP) were normal in both patient groups with or without relapses as mentioned in Table 4.

The study revealed the results of DEXA scan in RRMS study groups according to state at presentation (with relapse or without relapse in the last 6 weeks), in which the result was normal in 5 (33.3%) in patients with relapse and in 6 (40.0%) in patients without relapse , and osteopenic range in 8 (53.3%) in patients with relapse and in 6 (40.0%)in patients without relapse , and osteoporotic range in 2 (13.3%) in patients with relapse and in 3 (20.0%)in patients without relapse (p value = nonsignificant),as mentioned in Table 4.

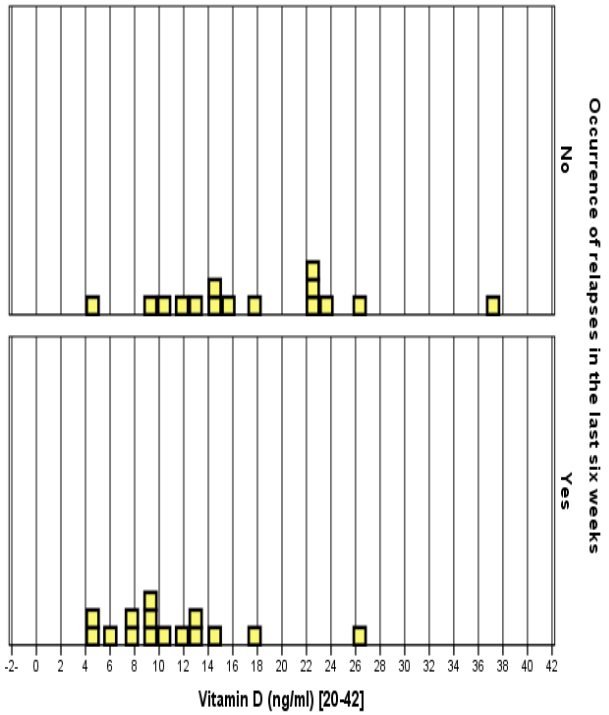

Figure 1: Shows distribution of vitamin D measurement in RRMS study group according to state at presntation (with relapse or without relapse in last 6 weeks).

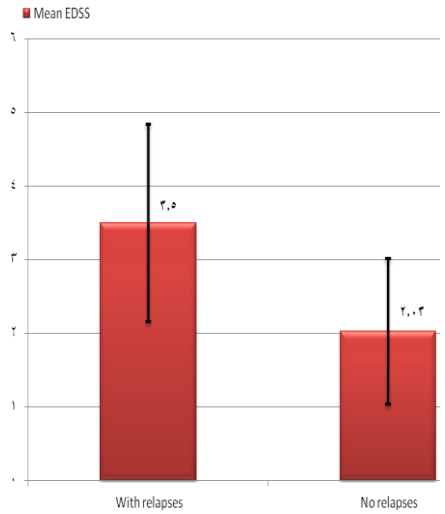

Figure 2: Shows mean of EDSS measurement in RRMS study group according to state at presntation (with relapse or without relapse in last 6 weeks).

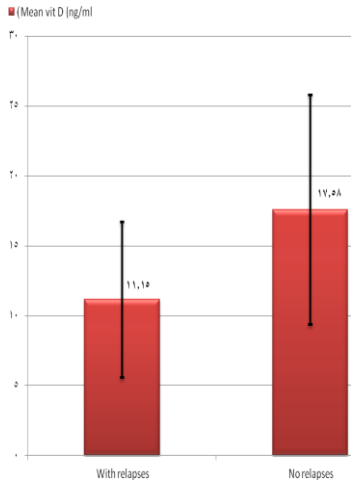

Figure 3: Shows mean of vitamin D measurement in RRMS study group according to state at presntation (with relapse or without relapse in last 6 weeks).

Table 6: Shows correlations of variable parameters with EDSS in patients group study.

Correlations		EDSS of Patients
Age (years)	r	0.258
	P	0.169
Duration of first symptoms (years)	r	0.483**
	P	0.007
Number of relapses in last 2years	r	0.543**
	P	0.002
Vitamin D (ng/ml)	r	0.039
	P	0.836
PTH (pg/ml)	r	0.222
	P	0.239
ALP (IU/L)	r	0.052
	P	0.786
DEXA scan	r	-0.185
	P	0.326

*Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level.

Table 6 shows that there is no correlation between the age of the patients,vit.D level, other related parameters (PTH, ALP, DEXA scan) with EDSS in patients group study (r correlation coefficient = non significant).

There is direct strong correlation of both duration from first symptom , number of relapses in last 2years with EDSS in patients group study (r correlation coefficient = significant).

DICUSSION:

There aremany studies on MS ; dealing with different aspects of the disease. This study and analysis sought to establish the association of vit.D metabolite levels with relapse rate and disability in patients with RRMS.

The striking difference in prevalence of MS and some other autoimmune diseases as a function of latitude has implicated vit.D status as a determinant of risk [16]. It has been estimated that

1 billion people worldwide have vit.D deficiency or insufficiency [17].In studies in countries around Iraq , 30 to 50% of children and adults had 25(OH)D3 levels under 20 ng per milliliter [7-8-9].

This study revealed that vit.D was low for both patients and controls but did not reach statistical significance probably because small sample size. These results show agreement with some previous studies by Van der Mei et al, 2007 [12], Soilu-Hanninen et al, 2008 [14] , they found high prevalence of vit.D deficiency in both MS cases and controls.

This study revealed that DEXA scan was subnormal in patients as compared to controls. It was statistically significant. This finding shows agreement with some previous studies by Ozgocmen et al, 2005 [19], Nieves et al, 1994[20].

In our study, we did find significantly lower 25(OH)D level in patients with relapse compared with patients without relapse in the last 6 weeks.

Our results show agreement with some previous studies by Soilu-Hanninen et al, 2008 [14], Correale et al, 2009 [21]. Similarly, researchers working in Tasmania reported a inverse relationship between relapses and estimated serum 25(OH)D [22].An inverse relationship was also observed between serum 25(OH)D levels in Tasmanian RRMS patients and risk of relapse, with each 10 nmol/l increase in 25(OH)D resulting in a 12% decrease in relapse risk [23]

Also, amongst patients in the USA with pediatric-onset MS or clinically isolated syndromes, vit.D status predicted subsequent rate of relapse: Each 25 nmol/l increase in seasonally adjusted 25(OH)D concentrations predicted a 34% decrease in subsequent relapse rate [24].

Also, our results show agreement with two Finnish studies [24] reported that mean serum 25(OH)D concentrations were lower during relapses than remission.

This study, we did find higher PTH levels in patients with relapse compared with patients without relapse in the last 6 weeks. It was statistically significant. This finding shows agreement with that of Hanninen et al, 2008 [14].

This study, we did find correlation between duration of first symptom (years) and EDSS in patients group study, It was statistically not significant. Also, we did find correlation between number of relapses in the last 2 years and EDSS in patients group study but it was statistically not significant. Our results show agreement with previous study by Tremlett et al, who showed the importance of early relapse to early, but not late, disability as measured by the EDSS [27].

Lastly , we didn't find any correlation between vit.D level and EDSS in patients group study and this is probably because of small sample size. This findindg not correlate with some previous studies that show inverse relationship of vit.D with MS disability by Van der Mei et al, 2007 [12],Soilu-Hanninen et al, 2008 [69] and Correale et al, 2009 [21].

CONCLUSION:

There is low circulating level of 25-hydroxvitamin D in RRMS patients, especially during relapses,also no correlation between vitamin D level and EDSS in patients with RRMS.The RRMS is associated with reduced bone mass and higher frequency of osteoporosis.

Recommendations:

We recommend monitoring of vitamin D levels in patients with RRMS that may predict the subsequent rate of relapse. We recommend vitamin D supplementation for patients with RRMS and vitamin D deficiency.

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