SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM, KERALA

Association of Serum 25 (OH) Vitamin D3 Concentration with Multiple Sclerosis

Thesis submitted in partial fulfillment of the rules and regulations for

DM Degree Examination of

Sree ChitraTirunal Institute for Medical Sciences and Technology

By

Dr. Pande Aniket Chandrakant

DM Neurology Resident

Month and Year of Submission: October 2015

Department of Neurology

Sree Chitra Tirunal Institute for Medical Sciences and Technology

Thiruvananthapuram

2013-2015

DECLARATION

I, **Dr. Pande Aniket Chandrkant**, hereby declare that this project was undertaken by me under the supervision of the faculty, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology

Thiruvananthapuram **Dr. Pande Aniket Chandrkant**

Date: 01/10/2015

Forwarded

The candidate, **Dr. Pande Aniket Chandrkant**, has completed the project under my guidance.

Thiruvananthapuram

Date: 03/10/2015

Dr.Abraham Kuruvilla (Guide)
Professor,
Department of Neurology,
SCTIMST.

Dr.Muralidharan Nair,(Co-guide)
Professor and Head,
Department of Neurology
SCTIMST

Forwarded

The candidate, **Dr. Pande Aniket Chandrkant,** has carried out the minimum required project.

Thiruvananthapuram

Date: 03/10/2015

Dr.Muralidharan Nair,

Professor and Head,

Department of Neurology

SCTIMST

ACKNOWLEDGEMENT

I take this opportunity to express my sincere gratitude to **Dr. Abraham Kuruvilla** and **Dr. Dr.Muralidharan Nair** for their expert guidance, constant review and kind help at each and every step of the study, without which the study would not have materialised.

I am thankful for their guidance, encouragement and valuable suggestions during the period of the study. I express my gratitude to nursing staff and medico-social workers in the Department of Neurology for their unwavering support for conducting the study. I express my sincere thanks to **Dr. Sankara Sarma**, Professor, Achutha Menon Centre for Health Science Studies for helping me with the statistical analysis of this study. I am extremely thankful to my colleagues in the Department of Neurology for their support and positive criticisms which were valuable during the course of the study. Last but not the least, I extend my gratitude to all my patients and their primary caregivers who willingly participated in this study.

Dr.Pande Aniket Chandrakant

Senior Resident

Department of Neurology

SCTIMST, Trivandrum, Kerala.

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INTRODUCTION

Multiple sclerosis is an autoimmune disease associated with inflammation of CNS, leading to formation of focal plaque of demyelination and degeneration of white matter as well as grey matter of the brain and spinal cord.[1] Disease usually begins with a relapsing and remitting course (RRMS), in some individuals followed by a secondary progression (SPMS). Few patients may have uninterrupted progression from disease onset i.e. primary progressive MS.

The etiology of MS is complex and is combination of multiple genes and their interaction with environmental factors.[3, 4, 5] Observational studies have suggested that Vit D(Vitamin D), infection with EBV and cigarette smoking are proposed environmental risk factors.[6] One noticeable feature of MS epidemiology is that increase in latitude ((φ) geographic coordinate) has positive correlation with increasing prevalence and incidence of MS.[7, 8]

Some studies have suggested sunlight and vitamin D (Vit D) levels in the etiology of MS.[10-13] Vit-D after activation (1, 25-OH Vit-D), has major role in bone metabolism and has significant immunomodulatory actions, leading to decreased proinflammatory pathways and probably has protective role in various autoimmune diseases.

REVIEW OF LITERATURE

Vitamin D group compounds are fat soluble secosteroids (seco meaning broken). They have multiple actions in the body which include role in Ca pathways, neuromuscular function, immunomodulatory function.[14]

The recent discoveries suggested that most of the cells in the body have a receptor for vit D, Several cells possess enzymes that can convert the prohormone to activated form of Vit D, which provided new understanding in function of this vitamin. Most important is reduction in the risk of chronic diseases, like autoimmne diseases, infectious diseases and cardiovascular diseases.[15]

Vit D exist in two major forms (prohormone form). [16, 17]

i) Cholecalcifarol (Vit-D3) animal source

ii) Ergocalciferol (vit D2) plant source

The activated forms of Vit- D3 are supposed to be much more biologically active than those of D2. In human being, major source of vit D is UV-B ray exposure, minimum contribution from diet, fortfied food and nutitional supplementation. Sun exposure for about twenty minutes produces equivalent to ingestion of at least 10,000 IU.[19]

Vitamin D metabolic pathway :-

In the body, cholecalcifarol / ergocalcifarol hydroxylated in hepatocytes to form 25-hydroxy Vit-D (25(OH) Vit-D) the storage form of vit D found in circulation. This metabolite again undergoes hydroxylation (1-α hydroxylase enzyme) in renal and other tissues to form 1, 25-dihydroxy Vit D3 ; the activated form of vit D. Vit-D after activation has characteristically very short $T_{1/2}$. Major vit D metabolite is 25(OH) Vit-

D and this is the commonly used form for assessing vit D status of a particular individual.[20]

Vitamin D and immune function

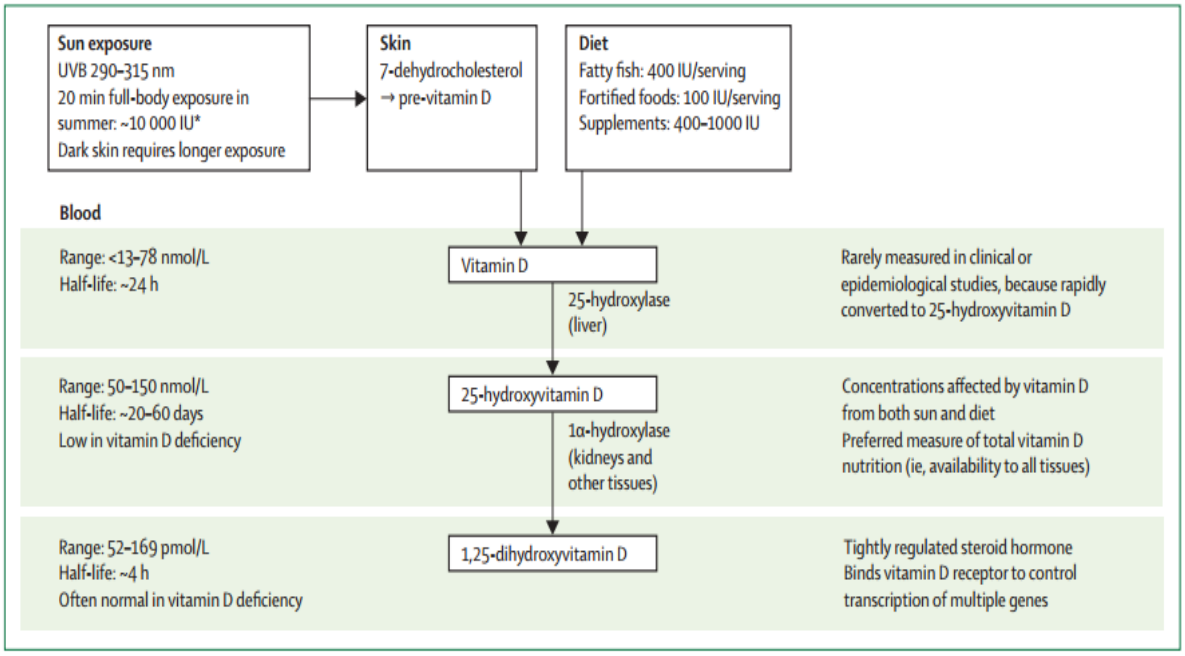
Most activation of Vit- D occurs in the renal cells and cells related to immune system including APCs (antigen-presenting cells) and monocyte/macrophage resulting in local production of activated Vit-D having both autocrine and paracrine functions.

i) Local 1, 25(OH) 2D have autocrine as well as paracrine functions.

ii) Most of the immune system cells have VDR[29]

iii) Active form of vit D acts on various immune cells and has immunomodulatory action[22]

Figure 1 :- Sources and metabolism of Vitamin D[52]



Vitamin D effect on innate immunity

The vitamin D facilitate innate immunity in vitro (" literally "in glass") and in vivo (Latin for "within the living") by increasing differentiation of macrophage precursors[24] and enhancing chemotaxis[24, 25]. Leading to more effective destruction of pathogens. The vit D also provides a check on uncontrolled production of inflammatory mediators.

Cell-mediated immunity

In vitro studies have shown that Vit- D influences the complex interaction between immune cells antigen presentation to T cells.[29] Vit D reduces maturation of antigen presenting cells[30] and decreases antigen presentation to T cells. Latest studies have suggested that Vit-D results in up-regulation of immunosuppressive T regulatory cells,[33,34] reduces interferon-gamma production.[35]

Evidence that link vitamin D to MS

Relation between latitude gradient and MS prevalence was initially described in 1920s[36] and association with sunlight was proposed in the 1960s[37].In both northern and southrn hemispheres lattitude gradient is observed, increasing lattitude is associated with rise in MS prevalence.[38] Increase in distance from the equator leads to increased atmospheric filtering of UVB radiation due to the oblique angles of the sun's rays at higher latitudes.[62] Less exposure to UV B leads to decreased production of vit D, UV rays also supposed to have immune modulatory properties.

Effect of Month of birth

It has been seen that people with MS are more commonly born in month of November in the southern and in May in northern hemispheres, as compared to the general population.[42] Association was seen with UV radiation exposure in Ist trimester of pregnancy[43] suggesting that this may be related to less maternal Vit- D due to less ambient UV in winter.

Vitamin D or UV radiation?

Recent studies have suggested that Vit- D deficiency and less exposure to sunlight (present or past) were associated independently with more risk of a demyelinating event [44] and this is observed in animal model of multiple sclerosis also.[45] Vit D (active form) has immunomodultory properties. Protection against autoimmune diseases with adequate vit D level can be postulated. UV radiation probably have similar immunomodulatory role. Evidence suggest that UV-related immunosuppression is independent of vit D action. Clear differentiation between immunomodulatory effects related to UV radiation vs local or systemic alterations in active Vit-D form is complex.

Genetic factors

Genes have effect on metabolism of Vit-D and skin complexion, both of which can affect circulating storage form of Vit-D level.[52] Genetic differences in vit D receptor and other genes modulate vit D action on the immune cells.[48] At present vitamin-D-related genes or genetic interactions related to vit D in determining MS risk is poorly understood. The HLA- DRB1*1501 haplotype is strong genetic predictor of MS risk, which may be modified by interactions with other HLA-DR haplotypes.[49]

Other genetic factors

If role of Vit- D in MS etiology is postulated, variations in genetic expression in Vit D pathway may also be associated with differential MS risk in different individual. But the relation between MS and VDR polymorphisms is not consistent.

HYPOTHESIS AND OBJECTIVES

OF THE STUDY

Hypothesis

i) Low Vitamin D level is associated with multiple sclerosis (MS).

ii) Low vitamin D level correlates with disability, disease severity and disease relapses.

Aims and objectives.

i) To evaluate whether vitamin D level is associated with multiple sclerosis (MS).

ii) To evaluate whether vitamin D level correlates with disability [measured by the Expanded Disability Status Scale (EDSS)] and Disease severity

iii) To evaluate whether vitamin D level is associated with disease relapse

MATERIALS AND METHODS

SUBJECTS AND METHODS

The study was a hospital based case control study. The subjects were selected from among the patients attending the neuromuscular disorder clinic and inpatient of a single tertiary center (Sree Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram).

Study period:

The study was conducted over a period of 21 months from January 2014 to September 2015.

Ethical considerations:

The study was approved by the Institute Ethical Committee. Written informed consent was obtained from all the subjects participating in the study. The informed consent procedure was done according to the guidelines provided in the Declaration of Helsinki and the ICH E6 Guideline for Good Clinical Practice.

Methodology:

Consecutive Patients fulfilling International Panel diagnostic criteria[54] [2010 Revisions to the McDonald Criteria] for MS were selected from January 2014 to September 2015. Written informed consent was obtained from all the cases and controls. Age and sex matched healthy unrelated controls recruited. Blood sample collection for Vitamin D level was done upon enrollment into the study. Blood sample of cases and age and sex matched controls was selected at the same time.

Detailed history was obtained and neurological examination was done at the time of drawing blood. Disability at the time of blood draw was measured by the Expanded Disability Status Scale (EDSS) and disease severity was assessed.

Severity of their disease was categorized into Mild, Moderate and Severe.

	EDSS
Mild	≤3
Moderate	3.5 - 5.5
Severe	≥ 6

Vit D concentration was measured with CMIA (carbonyl metallo assay)

Vit D status [55] was determined as.

Vit D Status	
Normal	> 30 ng/ml
Insufficient	20-30 ng/ml
Deficient	<20 ng/ml

Inclusion criteria

- Cases includes patient fulfilling International Panel diagnostic criteria for diagnosis of multiple sclerosis[54].
- Controls included healthy individuals not receiving any medication or dietary supplements, age and sex matched with cases.

Exclusion criteria

- Not willing to participate in the study
- Patients taking Vit D supplementation, conditions predisposing to hypercalcemia , nephropathy or renal insufficiency, liver diseases.
- Subject is diagnosed with another etiology causing white matter disease

Relapse was defined[56] as the acute or subacute appearance or reappearance of a neurological abnormality (that lasts at least 24 hours), immediately preceded by a improving, stable or slowly progressive neurological state in last 30 days, with no fever, known infection, concurrent steroid withdrawal, or externally derived rise in body temperature.

Statistical analysis:

Data analysis was performed using SPSS ver. 22.0. Quantitative variables were described by mean and SD, Qualitative variables were described by frequency distribution. Between group comparison of quantitative variables independent sample t test and that of qualitative variables chi- square test was used. A p value of 0.05 was taken as level of significant.

RESULTS

Demographic data

In our study we enrolled total 60 cases and 60 controls. Mean age of cases was 34.41 years (S.D.-12.3 , range 18 - 66). Mean age in male patients was 32.38 years (S.D.-12.49) while in female patients was 35.51 years (S.D.12.44). Cases and controls were comparable.

Table 1 :- Age distribution of cases and controls

Age	case		Control		**Total**	
	N	**%**	**N**	**%**	**N**	**%**
<25	19	31.7	18	30.0	37	30.8
26-35	16	26.7	18	30.0	34	28.3
36-45	13	21.7	12	20.0	25	20.8
>45	12	20.0	12	20.0	24	20.0
Total	60	100.0	60	100.0	120	100.0

$\chi^2=0.185$ $df= 3$ $p= 0.980$

Chart 1 Age distribution of cases and controls (%)

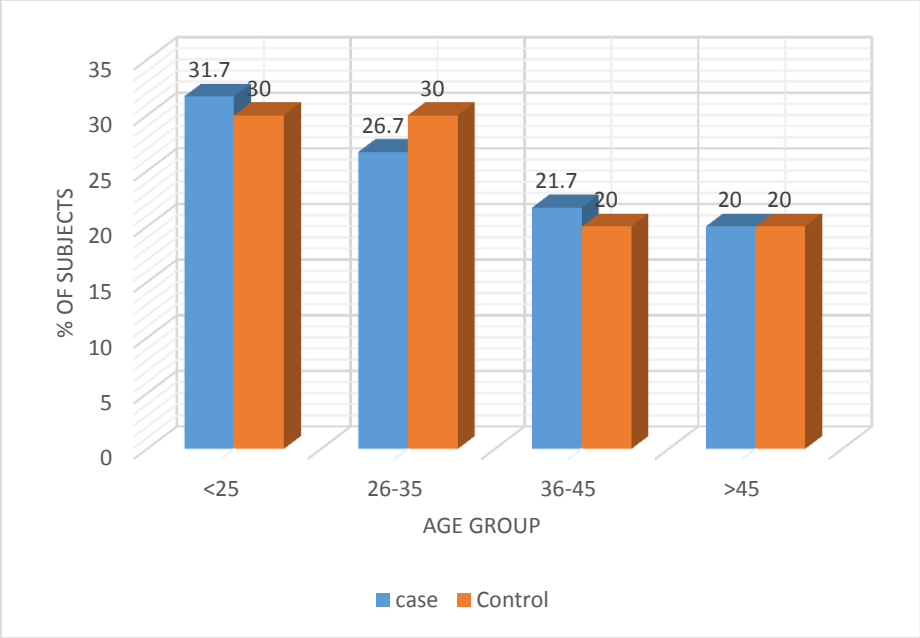
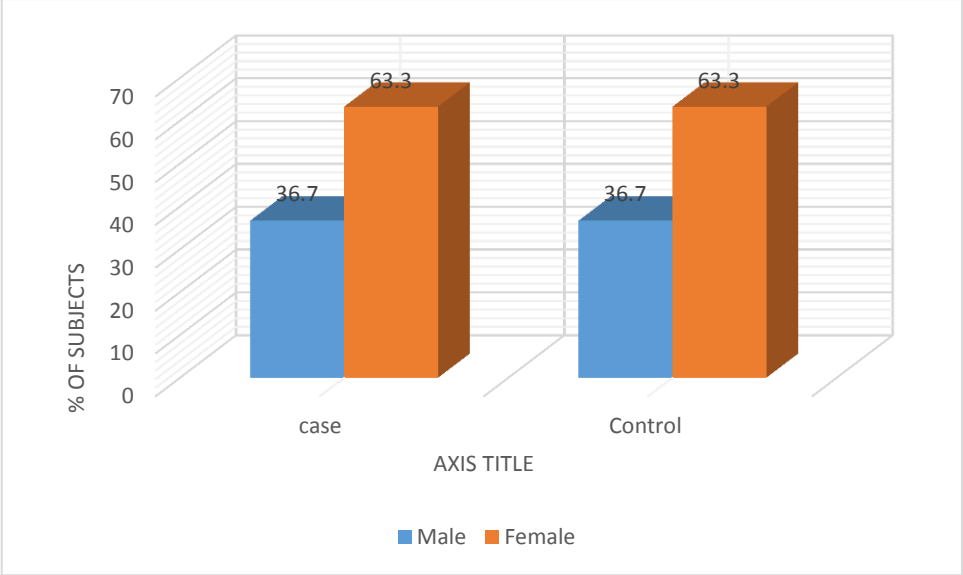


Table 2 :- sex distribution

Sex	case		Control		**Total**	
	N	**%**	**N**	**%**	**N**	**%**
Male	22	36.7	22	36.7	44	36.7
Female	38	63.3	38	63.3	76	63.3
Total	60	100.0	60	100.0	120	100.0

χ^2=0.000 df= 1 p= 1.000

Chart 2:- sex distribution

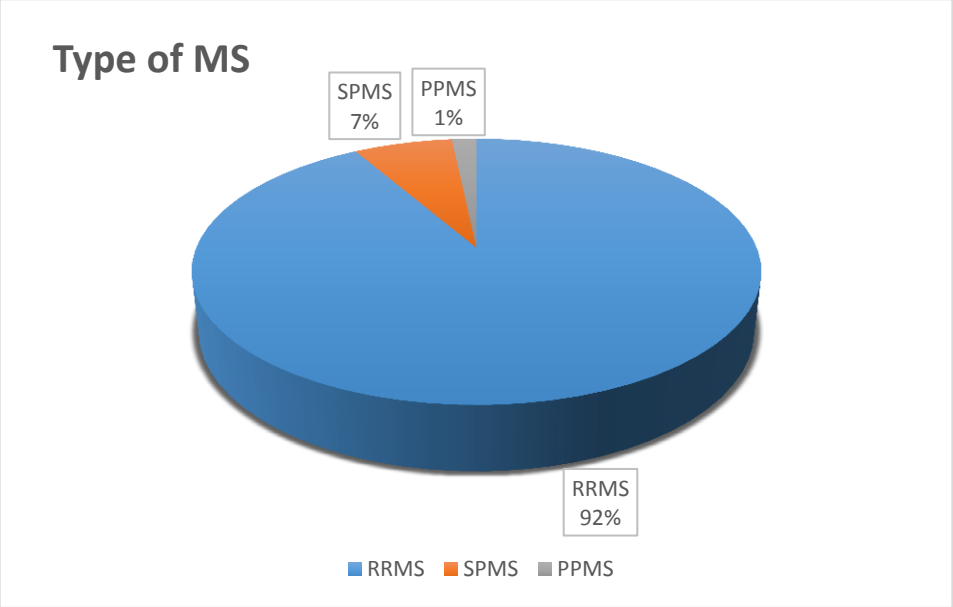


Controls were age and sex matched, In the study population number of males were 22 and female 38 in each group (cases and controls) and the groups were comparable. Almost 2/3 of the cases were females.

Table 3 :- Type of MS

RRMS	55 (91.66%)
SPMS	4(6.66%)
PPMS	1(1.66%)

Chart 3 :- Type of MS

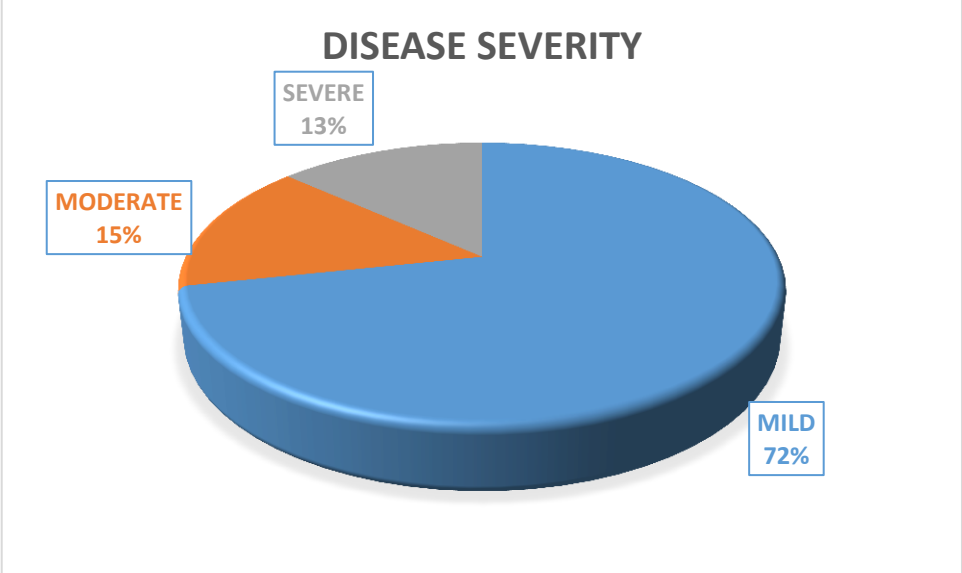


In the study group majority of the patients were of RRMS group 55/60 (91.66%), 4 (6.66) SPMS and only one (1.66%) patient was of PPMS type.

Table 4 :- Expanded disability status scale score and disease severity

Disease severity	MILD	MODERATE	SEVERE
EDSS	<3	3.5-5.5	>6
Number of patients	43	9	8

Chart 4 :- Expanded disability status scale score and disease severity



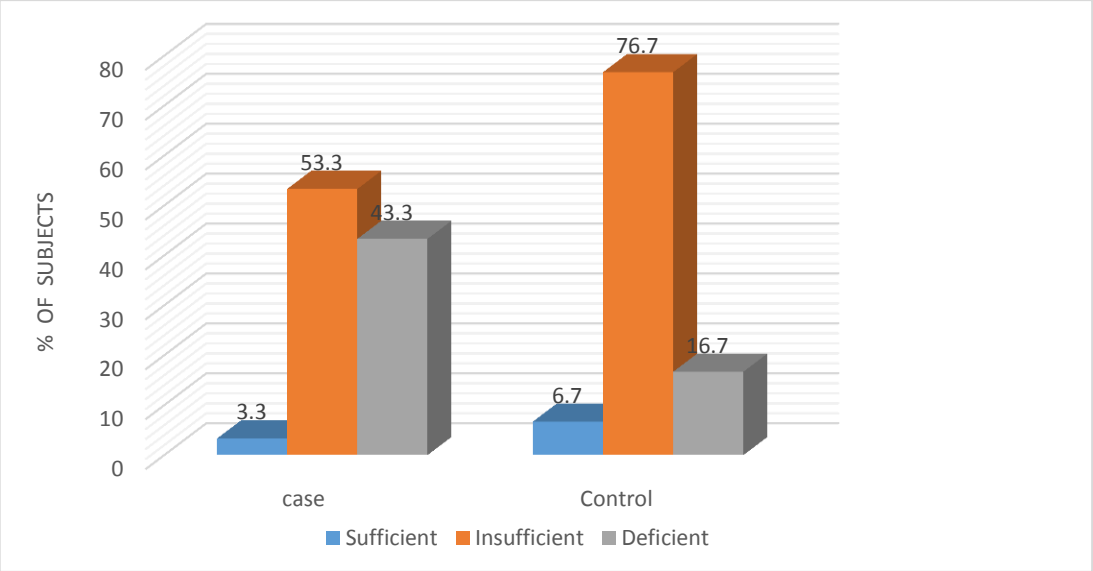
Case were classified according to the severity of the disease as measured by EDSS into 3 groups, Majority of the patients [n=43 - 72%] had Mild (EDSS <3) disease, moderate and severe disease in 9(15%) and 8 (13%) cases respectively

Table 5 :- Vitamin D status

Vitamin d status	case		Control		**Total**	
	N	**%**	**N**	**%**	**N**	**%**
Sufficient	2	3.3	4	6.7	6	5.0
Insufficient	32	41.1	46	58.9	78	65.0
Deficient	26	72.2	10	27.8	36	30.0
Total	60	100.0	60	100.0	120	100.0

χ^2=10.291 *df*= 2 ***p*= 0.006**

Chart 5 :- Vitamin D status

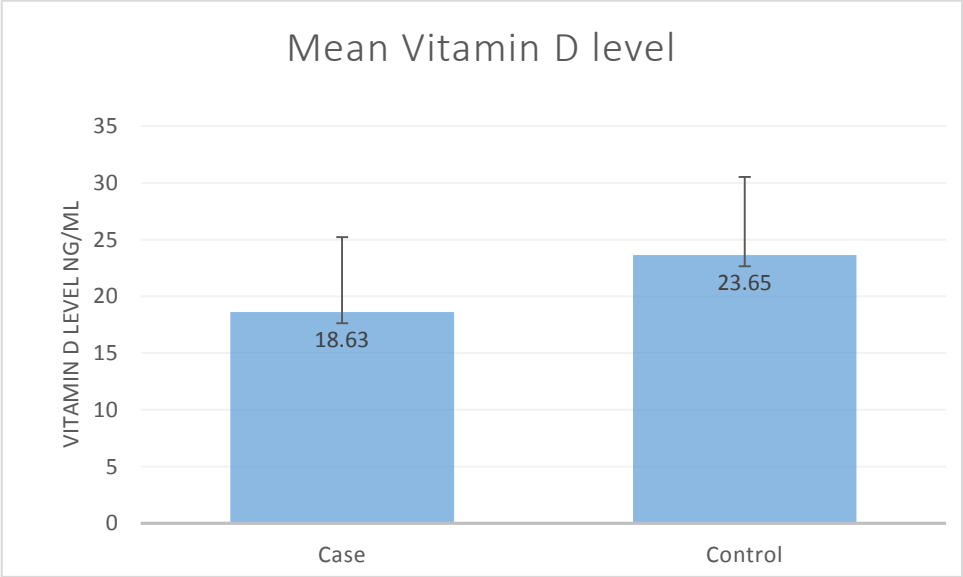


Vitamin sufficiency was seen in only 2 cases and 4 controls. Insufficient vitamin D levels were seen in 32 (41%) cases and 46 (58.9%) controls. Vitamin D deficieny (< 20) was seen in 26 (72.2%) cases and 10 (27.8%) controls. Vitamin D deficiency was significantly more common in cases than controls and vitamin D insufficiency was more common in controls (*p*-value 0.006).

Table 6 :- Mean vitamin D level

	N	Vitamin D level		t	p
		Mean	sd		
Case	60	18.63	6.58	4.081	<0.001
Control	60	23.65	6.87		

Chart 6 :- Mean vitamin level



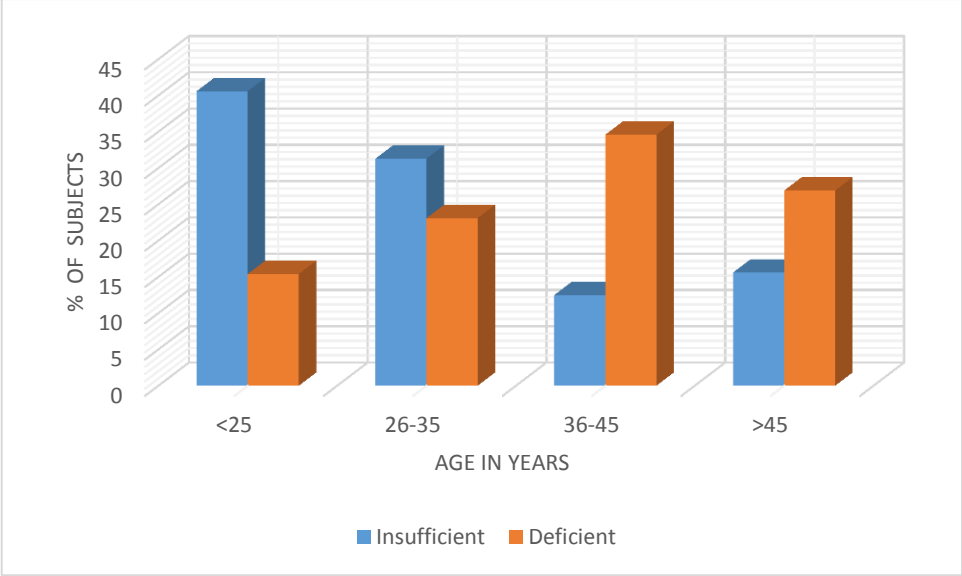
Mean vitamin D level in cases was 18.63 (Vitamin D deficiency) and that in controls was 23.65 (vitamin insufficiency), which was statistically significant (*p* value < 0.001).

Cases had significantly low vitamin D than controls.

Table 7 :- Vitamin D status in different age group

Age	Vitamin D status				Total	
	Insufficient		Deficient			
	N	%	N	%	N	%
<25	13	40.6	4	15.4	17	29.3
26-35	10	31.3	6	23.1	16	27.6
36-45	4	12.5	9	34.6	13	22.4
>45	5	15.6	7	26.9	12	20.7
Total	32	100	26	100	58	100

Chart 7 :- Vitamin D status in different age group



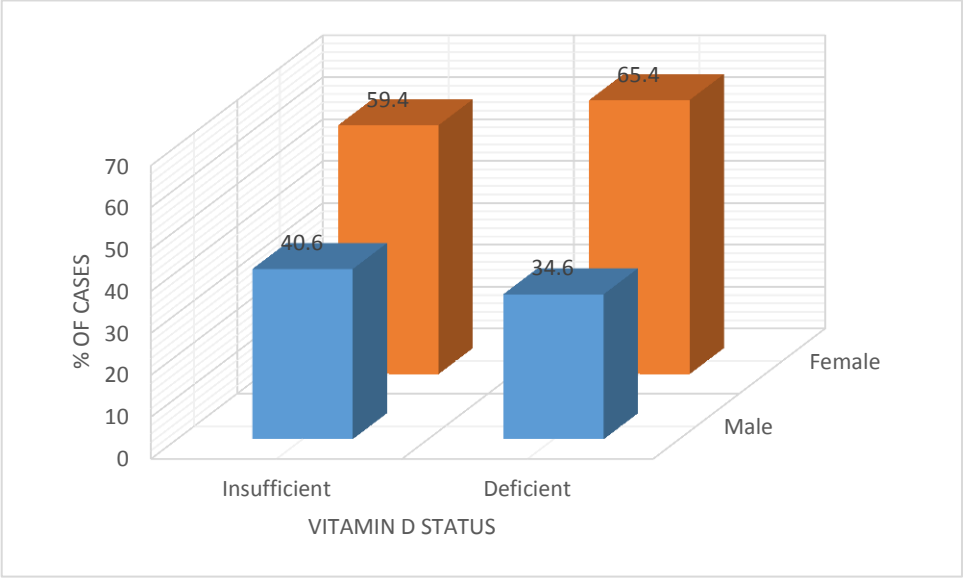
Vit D insufficiency was seen in all age groups, in age group < 25 years 13 cases (40.6%) , 26-35 years 10 cases (31.3%), 36-45years 4 cases (12.5%) and > 45 years 5 cases (15.6). Vitamin D deficiency was more common in age group 36-45 years, 9 cases(34.6%) were deficient In other age groups < 25 years 4 cases(15.4%),26-35 years 6 cases (23.1%) and > 45 year group 7 cases (26.9%).

Table 8 :- Vitamin D status and gender

	Vitamin D status				Total	
	Insufficient		Deficient			
SEX	N	%	N	%	N	%
Male	13	40.6	9	34.6	22	37.9
Female	19	59.4	17	65.4	36	62.1
Total	32	100	26	100	58	100

$\chi^2 = 0.220$ $df = 1$ $p = 0.639$

Chart 8 : - Vitamin D status and gender (%)

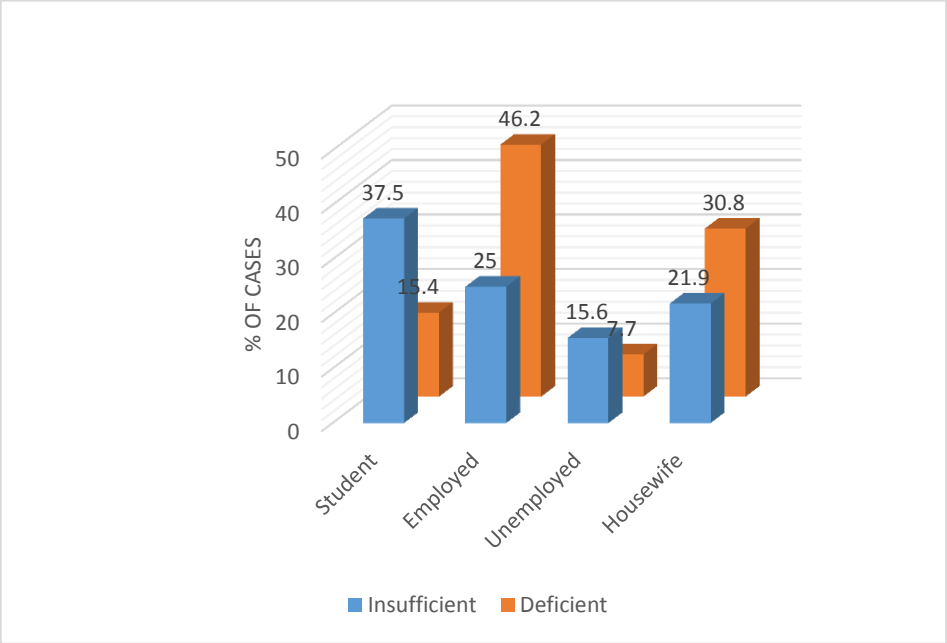


Vitamin D insufficiency as well as deficiency was more common in females but the difference did not reach statistical significance.

Table 9 :- Vitamin D status and occupation

Occupation	Vitamin D status				Total	
	Insufficient		Deficient			
	N	%	N	%	N	%
Student	12	37.5	4	15.4	16	27.6
Employed	8	25	12	46.2	20	34.5
Unemployed	5	15.6	2	7.7	7	12.1
Housewife	7	21.9	8	30.8	15	25.9
Total	32	100	26	100	58	100

Chart 9 :- Vitamin D status and occupation



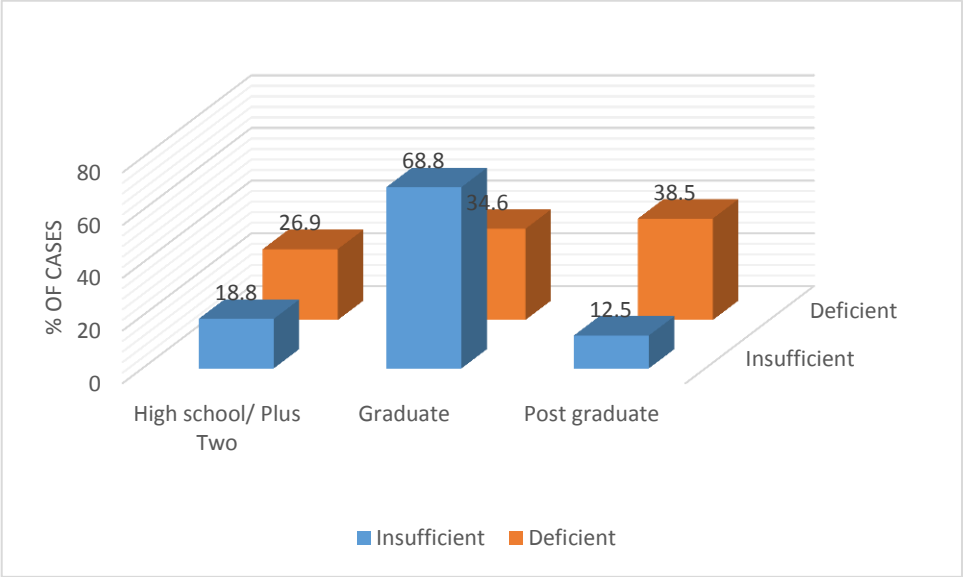
Vitamin insufficiency was more common in students and deficiency was more common in employed individuals. [Number of subjects were < 5 in some groups, statistical tests could not be done

Table 10 :- Vitamin D status and level of education

	Vitamin D status				Total	
	Insufficient		Deficient			
	N	%	N	%	N	%
High school/ Plus Two	6	46%	7	54%	13	
Graduate	22	71%	9	29%	31	
Post graduate	4	28%	10	72%	14	
Total	32		26		58	

$\chi^2 = 7.560$ $df = 2$ $p = 0.023$

Chart 10 :- Vitamin D status and level of education

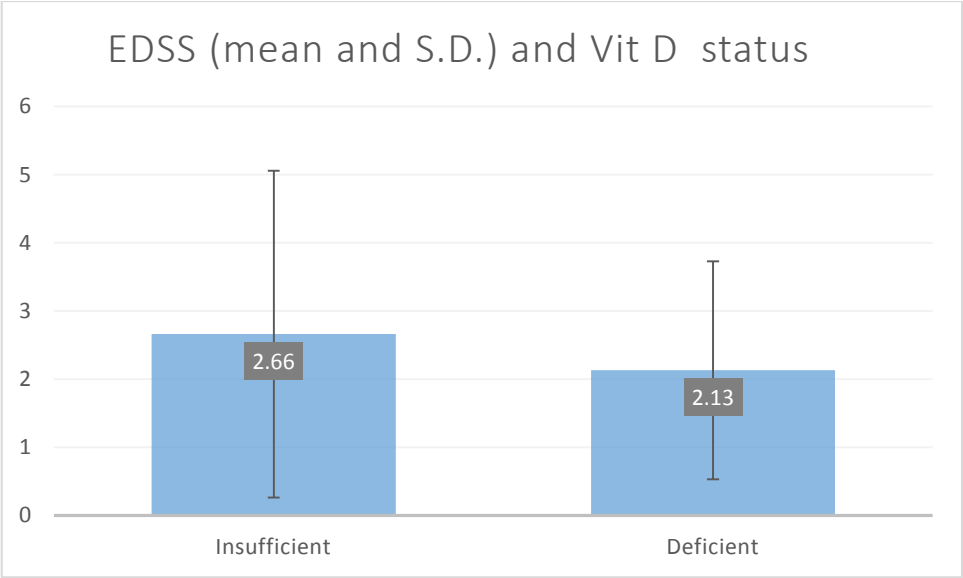


Vitamin D insufficiency was more common in graduate n= 22/31 (71%), Deficiency was more common in Postgraduate individuals10/14 (72%)

Table 11 :- Vitamin D and EDSS score

Vitamin D Status		EDSS		T	p
	N	Mean	SD		
Insufficient	32	2.66	2.40	.948	.347
Deficient	26	2.13	1.60		

Chart 11:- Vitamin D and EDSS score



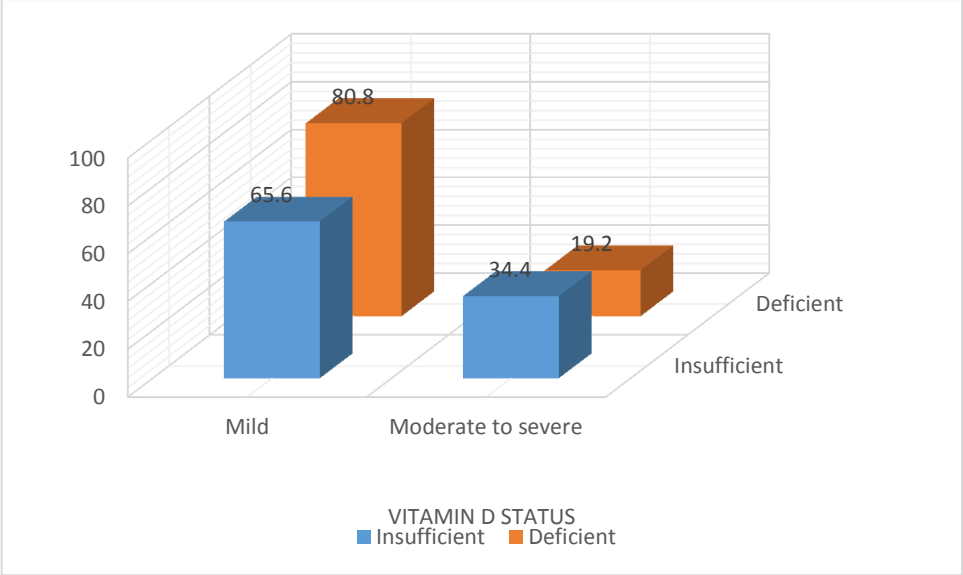
Mean EDSS in patients with Insufficient Vitamin D and deficient vitamin D was 2.66 (S.D. -2.4) and 2.13 (S.D. 1.6) respectively. In this study no significant correlation between Vitamin D status and EDSS score was seen.

Table 12 : - Vitamin D status and disease severity

Disease severity	Vitamin D status				Total	
	Insufficient		Deficient			
	N	%	N	%	N	
Mild	21	50%	21	50%	42	
Moderate to severe	11	69%	5	31 %	16	
Total	32		26		58	

$\chi^2 = 1.647$ *df =1* *p=0.199*

Chart 12 :- Vitamin D status and disease severity

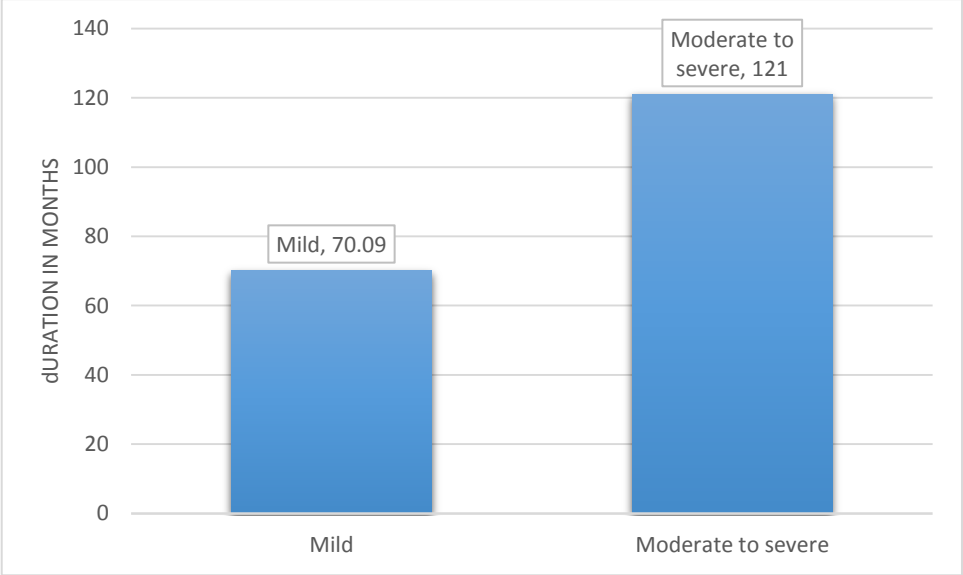


Mild disease was observed in 42 cases, half the cases had insufficiency and half had deficiency. Moderate to severe disease was seen in 16 cases, Vit D insufficiency and deficiency was seen 69% and 31% cases respectively.

Table 13 :- Severity of disease and disease duration.

Severity of disease		DISEASE DURATION		t	p
	N	Mean	sd		
Mild	43	70.09	67.97	-2.178	.033
Moderate to severe	17	121.00	109.49		

Chart 13 :- Severity of disease and disease duration.



Mean disease duration in patients with mild disease was 70.09 months (S.D.67.97) as compared to cases with moderate to severe disease 121 months (*p* value 0.033) which was statistically significant. The patients with moderate to severe disease had longer duration of disease as compared to patients with mild disease.

Table 14 :- Disease severity and age of symptom onset month

Severity of disease		AGE OF SYMPTOM ONSET MONTH		T	p
	N	Mean	SD		
Mild	43	333.40	137.31	.355	.724
Moderate to severe	17	319.88	120.03		

In cases with mild disability mean age of symptom onset was 333.4 month (27 year 9 months) and in cases with moderate to severe disability it was 319.8 months (26 years 7 months). The cases with early age of onset had more severe disease, but it was not statistically significant. There was no correlation between age of symptom onset and disease severity.

Table 14 :- Severity of disease and number of relapses

Severity of disease		NUMBER OF RELAPSES		t	p
	N	Mean	sd		
Mild	43	2.42	1.65	-1.933	.058
Moderate to severe	17	3.41	2.12		

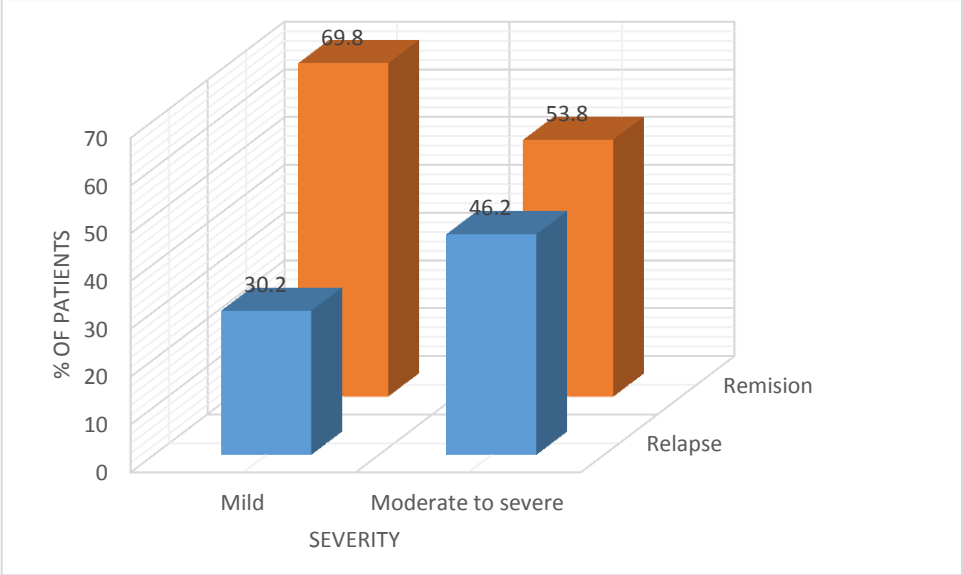
Mean number of relapses were 2.42 and 3.41 in cases with mild and moderate – severe disease groups respectively. Though the mean number of relapses were more in cases with more severe disease but difference was not statistically significant.

Table 15 :- Disease status and severity

Disease status	SEVERITY				Total	
	Mild		Moderate to severe			
	N	%	N	%	N	%
Relapse	13	30.2	6	46.2	19	33.9
Remission	30	69.8	7	53.8	37	66.1
Total	43	100.0	13	100.0	56	100.0

$\chi^2 = 1.129$ $df = 1$ $p = 0.288$

Chart 15:- Disease status and severity



In this study 43 cases had mild disease, out of which 13(30.2%) were in relapse and 30(69.8%) were in remission. In group with moderate to severe disease(n= 13) 6 (46.2%) were in relapse and 7 (53.8) were in remission. There was no significant correlation between severity of disases measured by EDSS and disease activity (relapse vs remission) [*p=0.288*]

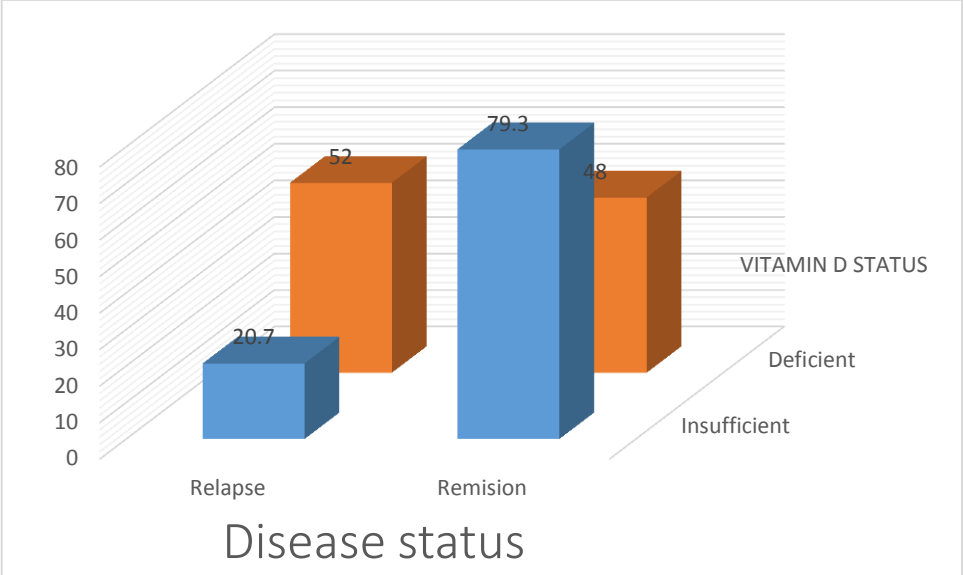
Table 16 :- Vitamin D and disease status

Disease status	Vitamin D status				Total	
	Insufficient		Deficient			
	N	%	N	%	N	
Relapse	6	31	13	69	19	
Remission	23	66	12	34	35	
Total	29	100	25	100	54	

In one patient in remission vitamin D level was normal (sufficient) Excluded from the table above

$\chi^2 = 5.771$ $df = 1$ $p = 0.016$

Chart 16 :- Vitamin D and disease status

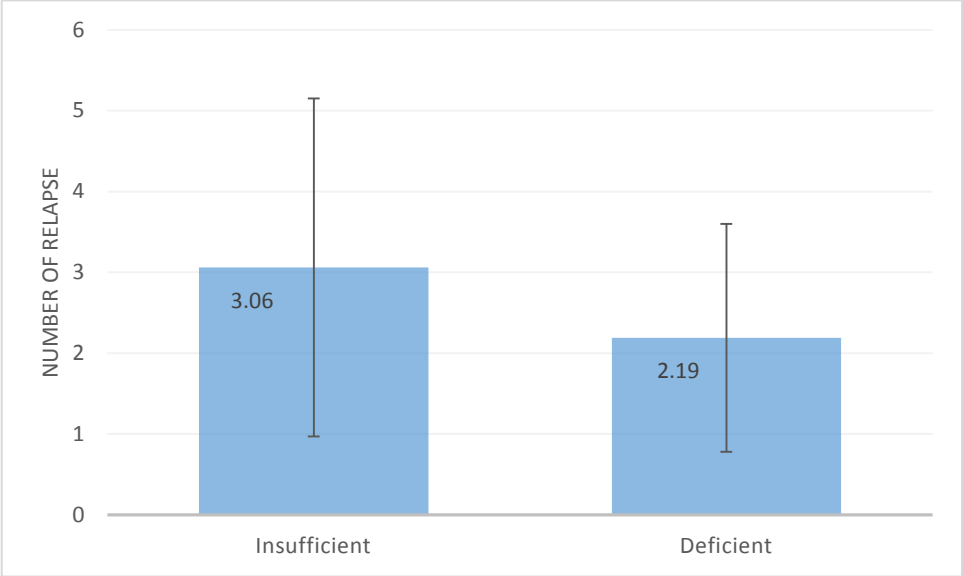


In this study total 19 patients blood sample was taken during relapse of multiple sclerosis and 36 patients during remission. One patient had sufficient levels of Vitamin D(excluded from analysis). In cases with relapse (n=19), 69% had deficiency and 31 had insufficiency. In cases with remission (n=35), 34.% had deficiency and 66 had insufficiency.There was significant association of Vitamin D deficiency with relapse and insufficiency with remission.

Table 17 :- Vitamin D and number of relapses

Vitamin D Status	number of relapses			t	p
	N	Mean	sd		
Insufficient	32	3.06	2.09	1.809	.076
Deficient	26	2.19	1.41		

Chart 17 :- Vitamin D and mean number of relapses

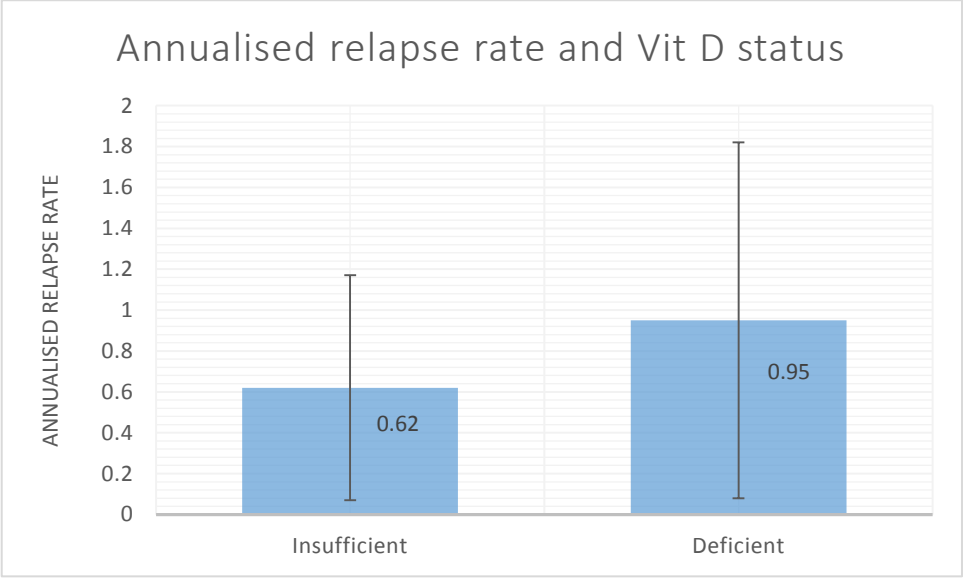


Mean number of relapses in Vitamin D insufficient and deficient individuals were 3.06 (S.D - 2.09.) and 2.19 (S.D.- 1.41) respectively.No statistical significance was observed.

Table 18 :- Vitamin D Status and Annualized relapse rate

Vitamin D Status		Relapse rate		T	p
	N	Mean	SD		
Insufficient	32	0.62	0.55	-1.767	.083
Deficient	26	0.95	0.87		

Chart 18 :-Vitamin D Status and annualized relapse rate



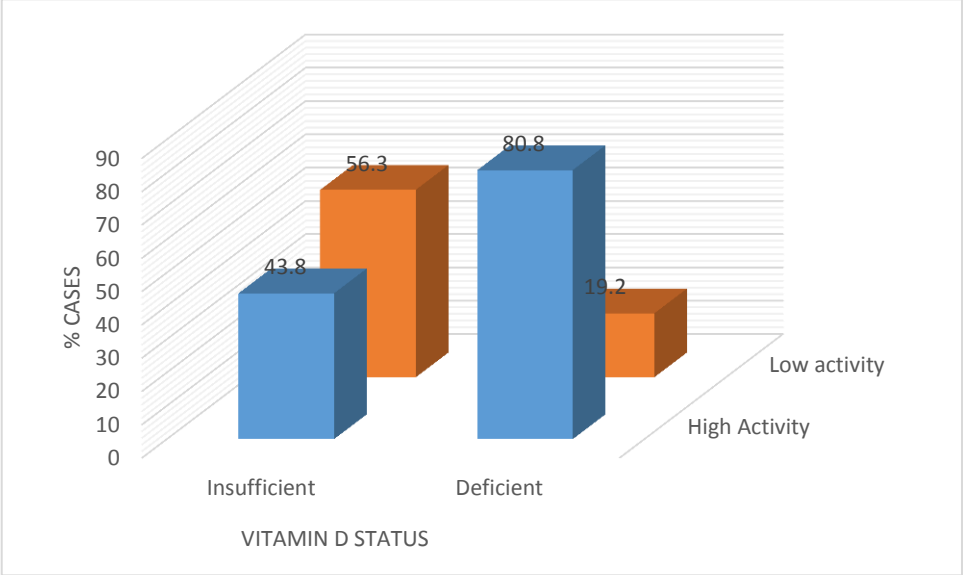
Mean annualized relapse rate in cases with Vitamin D insufficiency and deficiency was 0.62 (S.D.-0.55) and 0.95 (S.D.- 0.87) respectively. No statistically significant correlation was seen between the two groups (*p*- 0.083).

Table 19 :- Vitamin D and disease activity

activity group	Vitamin D status				Total	
	Insufficient		Deficient			
	N	%	N	%	N	%
High activity	14	40%	21	60%	35	58.3
Low activity	19	66 %	6	24%	25	41.7
Total	33	100	27	100	60	100

$\chi^2 = 8.251$ $df = 1$ $p=0.004$

Chart 19 :- Vitamin D and disease activity



In this study High activity disease was seen in 35 (58.3%) and low activity in 25 (41.7%) cases. In cases with high activity Vitamin deficiency was seen in 60% and insufficiency in 40% In cases with high activity Vitamin deficiency was seen in 34% and insufficiency in 66% Vitamin D deficient status was significantly associated with high activity group, insufficient status was associated.

DISCUSSION

The present study is a case control study to determine the Vit D status of cases with Multiple sclerosis and age, sex, timing of blood collection matched controls. We have randomly selected 60 cases from among the patients attending the neuromuscular clinic and inpatient in this institute and compared with 60 matched controls. The study has looked in to the level of Vit D in cases and general population (controls), assessing the relation between Vit D with disease severity and disease status (relapse/remission).

We have conducted this study in southern most part of India where Vit D deficiency is expected to be rare in view of geographical location near to equator, Fish as mainstay of diet which is rich source of Vit D

Demographic characteristics of the population and Vitamin D status

Two studies from india evaluated vit D and Multiple sclerosis. Lekha Pandit et al[57] studied 110 MS patients and 108 matched controls from 'Mangalore demyelination disease registry'. In this study mean age of cases was 35.4 (± 9.6) years, and 80% of the cases were females. In control group mean age was 36.1 ± 10.3 years and females were 79%. The median 25(OH)D levels were significantly low in MS patients (39.0 nmol/l) than in controls (46.5 nmol/l). Vit D deficiency was observed in 70% cases and 53.7 % controls. In vitamin D *insufficient group* no significant difference was seen between cases and controls. No correlation was observed between disease duration or EDSS and 25(OH) vit D levels.

Radhakrishnan Suresh Kumar et al[58] studied 26 MS cases, study population was from the state of Kerala in south India, of which 23 (88%) were females with age matched 202 controls. Mean age of cases was 32.6 years and controls was 37.5 years.

Mean vitamin D value for cases was 14.84 ng/ml and for controls were 18.55 ng/ml, Vitamin D levels were low in cases than controls.

J smolders et al[59] conducted Cross-sectional study of 267 MS cases from southern area of the Netherlands. At serum sampling clinical evaluation was done, EDSS scores calculated and Total relapses in 2 year period prior to collecting Vit D were counted. Mean age at serum sampling was 47.0 years (S.D.± 11.6) age at First symptom onset was 34.1 years (S.D. ± 10.1). In gender distribution number of Males were 66/267 (24.7%) and Female 201/267 (75.3%). On comparing with the RRMS patients , Vit -D was significantly low in cases with progressive form of MS. There was no genderwise difference in Vit-D levels. In the whole MS population, Vit D levels were negatively associated with EDSS score.

MS Barnes et al[60] in their case-control study performed in Ireland / UK measured concentrations of 25(OH)D in 29 individuals with multiple sclerosis and 22 controls (age, sex matched). They did not find any significant difference in Vit D between cases with MS and healthy controls. Significant difference in the Vit D metabolite level was observed in males and females, most marked in the MS group. Females had higher Vit D than men, but major limitation of this study was small sample size.

In the present study we have selected 60 cases and 60 age and sex matched controls. Out of 60 total 38 cases (63.3%) were females and rest 22 (36.7%) were males. Almost 2/3 of the cases were females, similar to gender distribution seen in other studies. Vitamin D insufficiency as well as deficiency was more common in females, but did not reach statistical significance. Mean Vitamin D level in cases was

18.63 (±6.58) and controls 23.65 (±6.87), Low levels of vitamin were seen in cases as well as controls, Vitamin D levels were significantly lower in cases than controls. On comparing the education and occupation with vitamin D status, Vitamin D insufficiency was more common in students and graduates, deficiency was more common in Postgraduates and employed individuals.

Mavila Veetil[61] et al studied Vit D levels among 82 doctors and 50 healthy controls in tertiary referral center in Kerala, India. Vit D deficiency (<20 ng/ml) was seen among 93.9% of the study group and 40% of the control group, suggesting high prevalence of vit D deficiency among doctors. Education and occupation related low vit D levels were probably related to less exposure to sunlight.

Being a tropical country it was strongly believed that low Vit D status is very rare in India. Our study population was from the state of Kerala in south India, our population has very low chance of Vit D deficiency due to proximity to the equator and Vit D rich fatty fish as mainstay of diet. Vit D deficiency increases with increase in distance from the equator because of increased atmospheric filtering of UVB radiation due to the oblique angles of the sun's rays at higher latitudes[62], our study population was mainly from southern most part of the kerala which is nearer to equator. Our study suggest that the low Vit D is very common in cases as well as controls. However there are some factors in this population that may favour the development of vit D deficiency. First is the colour of the skin and less sunlight exposure due to lifestyle changes. It has been shown that dark skinned individuals requires longer duration of sunlight exposure to produce similar amount of vit D than in fair skinned persons.[72] In our population most of the cases as well as controls had very less exposure to sunlight , due to education or occupation they spend most of the time indoor. In this study Vit D

deficiency was more common in females, but not statistically significant. Studies have shown that estrogen has role in vit D synthesis, with increased concentrations seen during puberty [63] and also during pregnancy[64], when MS symptoms are improved in many patients. JJ Kragt et al[65] found that higher levels of Vit D are associated with a lower MS incidence only in females. Animal studies suggest difference in Vit D metabolism in male and female animal, as seen in EAE; with Vit D supplementation resulting in delay in onset and less severity of the disease only in female mice but not in male mice.[66] If such genderwise difference proved, and showed in the CNS of human , this may explain more prevalence of MS in females and its relation to Vit D.

Disease severity and Vitamin D levels

Association of vitamin D with severity of multiple sclerosis has been studied in various studies in india as well as in other countries.

I.A.F.vanderMei et al[67] performed population based case-control study in Tasmania, Australia. This study included 136 cases and 272 community based matched controls. In cases, severe disability was strongly correlated with lower levels of Vit D. Cases with higher disability (EDSS ≥3) were more likely to have Vit D insufficiency than controls. Major strength of this study was that it was a population-based sample and not hospital based sample.

J.M. Gelfand et al [68] studied 25 (OH) Vit D levels in a cross-sectional sample including 339 African Americans with MS and 342 African Americans as control. There was no association between the MS severity and Vit D status. Ellen M. Mowry[70] et al in their EPIC study (described earlier) found higher Vit D level were associated with less disability on subsequent follow up, higher vit D levels were

correlated with less relapse risk; but this association was not statistically significant. One more important observation was; for disability, every 10ng/ ml higher Vit D level was associated with a 0.02- point lower EDSS score.

J Smolders et al[59] investigated the correlation between vit D concentration and MS severity as. In this Cross-sectional study, in 267 MS patient's blood was collected for 25(OH) D, they found that low 25(OH) D levels were associated with high EDSS-scores. Lekha Pandit et al[57] in their study from Mangalore compared vit D and disease charactristics , no relationship was observed between duration of MS or EDSS and 25(OH) D levels.

Masoud Nikanfar et al[71] conducted a case-control study, including 168 MS cases and 168 matched healthy controls in Tabriz, Iran. They did not find any significant correlation in EDSS score with vit D concentration, in this study there was significant association between EDSS score and disease duration. Cases with longer duration had more severe disease.

In our study mean disease duration was 84.6 months and mean EDSS was 2.4. There was no association between Vit D level and EDSS or severity of MS. When compared with some other studies where Vit D deficiency was associated with MS severity and EDSS , we did not find any statistically significant association. Our study population was small and this was not a prospective study, these may be the probable reason for this insignificant Correlation. Other factors may play role in determining immunomodulatory effect of Vit D, like genetic factors, skin colour. Genetic variations in VDR and other genes might influence the action of Vit D on the immune system.[52] Variations in Vit D related genes may also affect the MS risk, risk of relapses and

consequent disability, either directly or by modifying the effects of vit D. It is also possible that the biological consequences of a lower vit D status in darker pigmented populations might be different than in lighter pigmented populations.[72,73]

Degree of disability is affected by course of the disease. We found association between longer duration of the disease and disability. In our study the cases were hospital based [and not population based, like in some of the other study[67]] where cases are more likely to have greater disability and longer duration. Patients with severe disability are more expected to remain indoors (thus decreasing sunlight exposure), but in our study we did not find any correlation between EDSS (disease severity) and Vit D status

Vitamin D and disease relapse

RRMS is characterized by relapses, multiple factors may be related for a particular relapse in MS patients. Studies done in different parts of world have compared the relation of Vitamin D and MS relapse. Study by Lekha Pandit et al[57] fom india found significantly low vitamin D during relapse as compared to during disease remission. Steve Simpson et al[74] prospectively studied 145 cases from Southern Tasmania, Australia with RRMS. In this study Vit D concentrations were measured biannually, and relapses were assessed using survival analysis. Normal vit D concentrations were associated with a reduced hazard of relapse. There was linear relationship, with each 10nmol/l increase in Vit D concentration a 12% decrease in relapse risk was observed. They also proposed that clinically, increase in vit D concentration by about 50 nmol/l could reduce hazard of relapse of the disease by about 50%. There was no significant difference in vit D levels and relapses in patients with

high (>EDSS 4.5) or <low (EDSS 4.5) disability. Suggesting that this correlation was not driven by the mobility of the patients, active disease leading to less ambulation, less UVR exposure and less Vit D production.

Tessel F. Runia[75] et al in their prospective longitudinal study in 73 patients with RRMS measured 25(OH) Vit D concentration every 8 weeks. They studied correlation between 25 hydroxy Vit D concentration and exacerbation rates by poisson regresion (generalized estimating equations) with each serum Vit D level as time-dependent variable. Higher Vit D concentrations were associated with less exacerbation risk in RRMS, suggesting a favourable action of Vit D on disease course in MS. Ellen M. Mowry et al[70] in a retrospectively analyzed 110 pediatric MS cases and found that lower 25 OH- Vit D levels are associated with a significantly high subsequent relapse rate in pediatric-onset MS or CIS.

In our study in 19 cases blood sample was collected during relapse of multiple sclerosis and 36 cases during remission. In single case with remission levels of Vit D were sufficient. About 2/3 of cases with relapse (68.4%) had Vit-D deficiency, 2/3 cases with remission (65.7%) had insufficiency. There was significant correlation between Vit D deficiency with relapse and insufficiency with remission of MS. Mean annualized relapse rate was 0.62 and 0.95 in cases with insufficient and deficient group respectively, though ARR was more in cases with more severe deficiency the significance was not statistically significant. One of the major limitation of ARR was cases with very long duration of disease. While studying vit D and disease course in MS, issue of reverse causality is common. The possibility that the relapse may or may not be caused by lower vit D concentration, but that the low vit D levels may be due to increased disability that prevents person from spending time outdoors and decreases

sunlight exposure, which is the major source of vit D. We compared the disability of cases with relapse vs those who are in remission. We found that Moderate to severe disability (EDSS>3) which may impair ambulation was seen in 46.2 % cases with relapse and in 53.8% cases with remission, there was no significant difference between the two groups. In our cases and controls, cases could not give history of duration of exposure to sunlight, many of them had almost no exposure to sunlight. Considering the modulatory role of Vit D on cells with immune system, relapse of Vit D may be related to low vit D levels. Prospective studies with regular vit D level monitoring in same case during relapse and remission may provide further insight into the role of vit D in relapse of MS.

Vitamin D and disease activity

Murat Yildiz[77] et al in a cross sectional case control study in north-eastern Switzerland, 80 patients of RRMS were selected and divided into high disease activity and compared them to a group of patients who were clinically and MRI disease activity free (no relapse, clinical progression, no new MRI lesions -T2/ contrast enhancing over the past 2 years) (low activity group). They concluded that Vit D deficiency is common in cases and Vit D level was low in patients with high disease activity. In our study we similarly divided cases into high and low disease activity group. We found vit D deficiency significantly more common high activity group than low activity group (*p=0.004*).

CONCLUSIONS

1. Low vitamin D level was seen in cases as well as controls, multiple sclerosis patient have lower vitamin D level than control.
2. Low vitamin D level was seen in all age groups, both sexes, all educational and occupational groups.
3. No correlation between Vitamin D status and severity of the disease (as measured by EDSS).
4. No correlation between severity of the disease with age of symptom onset, number of relapses, relapse rate, disease status (remission vs relapse) at the time of enrollment into the study.
5. Cases with longer duration of the disease have more severe disease.
6. Vit D deficiency was more common in cases with relapse than remission.
7. Patients with high disease activity had vitamin D deficiency than those with low disease activity.

LIMITATIONS

1] This was not a prospective study, those who found to have low Vitamin D levels; we have started on Vitamin D supplement and cases were not further followed up for relapses.

2] Small sample size.

3] This was a hospital based sample and not population-based, where patients generally tend to have a higher disability and longer disease duration.

4] We have measured vitamin D levels after disease onset that could be affected by reverse causality.

REFERENCES

1] Lassmann H, Brück W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. Brain Pathol 2007; **17:** 210–18

2] Lublin FD, Reingold SC, and the National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Defi ning the clinical course of multiple sclerosis: results of an international survey. Neurology 1996; **46:** 907–11

3] Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol 2007;61:288e99.

4] Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707e17.

5] Trapp BD, Ransohoff R, Rudick R. Axonal pathology in multiple sclerosis: relationship to neurologic disability. Curr Opin Neurol 1999;12:295e302

6] Lazaros Belbasis, Vanesa Bellou, Evangelos Evangelou, John P A Ioannidis, Ioanna Tzoulaki Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses Lancet Neurol 2015; 14: 263–73

7] Alonso A, Hernan MA. Temporal trends in the incidence of multiple sclerosis: a systematic review. Neurology 2008;71:129–135.

8] Hammond SR, McLeod JG, Millingen KS, et al. The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle and Hobart. Brain 1988;111(pt 1):1–25

9] 4. van der Mei IA, Ponsonby AL, Blizzard L, Dwyer T. Regional variation in multiple sclerosis prevalence in Australia and its association with ambient ultraviolet radiation. Neuroepidemiology 2001;20:168–174.

10] van der Mei IA, Ponsonby AL, Dwyer T, et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ 2003;327:316.

11] Munger KL, Levin LI, Hollis BW, et al. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;296: 2832–2838.

12] Correale J, Ysrraelit MC, Gaitan MI. Immunomodulatory effects of Vitamin D in multiple sclerosis. Brain 2009;132:1146–1160.

13] Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology 2004;62:60–65.

14] Sarah Hewer a, Robyn Lucas b, Ingrid van der Mei c, Bruce V. Taylor Vitamin D and multiple sclerosis Journal of Clinical Neuroscience 20 (2013) 634–641

15] Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–81

16] Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 2006;84:694–7.

17] Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab 2004;89:5387–91

18] Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–8.

19] Holick M. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 1995;61:638S–45S.

20] Smith JE, Goodman DS. The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. J Clin Invest 1971;50:2159–67.

22] Alberto Ascherio, Kassandra L Munger, K Claire Simon Vitamin D and multiple sclerosis Lancet Neurol 2010; 9: 599–612

23] Boonstra A, Barrat FJ, Crain C, et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001;167:4974–80.

24] Xu H, Soruri A, Gieseler RK, et al. 1,25-Dihydroxyvitamin D3 exerts opposing effects to IL-4 on MHC class-II antigen expression, accessory activity, and phagocytosis of human monocytes. Scand J Immunol 1993;38:535–40.

25] Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-gammamediated antimicrobial activity of human macrophages. Sci Transl Med 2011;3:104ra102.
26] Adams JS, Vitamin D. As a defensin. J Musculoskelet Neuronal Interact. 2006;6:344–6.
27] Yim S, Dhawan P, Ragunath C, et al. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007;6:403–10.

28] Yuk JM, Shin DM, Lee HM, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6:231–43.

29] Lemire JM, Adams JS, Kermani-Arab V, et al. 1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity in vitro. J Immunol 1985;134:3032–5

30] Xu H, Soruri A, Gieseler RK, et al. 1,25-Dihydroxyvitamin D3 exerts opposing effects to IL-4 on MHC class-II antigen expression, accessory activity, and phagocytosis of human monocytes. Scand J Immunol 1993;38:535–40.

31] Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-gammamediated antimicrobial activity of human macrophages. Sci Transl Med 2011;3:104ra102.

32] van Halteren AG, van Etten E, de Jong EC, et al. Redirection of human autoreactive T-cells Upon interaction with dendritic cells modulated by TX527, an analog of 1,25 dihydroxyvitamin D(3). Diabetes 2002;51:2119–25

33] Yim S, Dhawan P, Ragunath C, et al. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007;6:403–10.

34] Yuk JM, Shin DM, Lee HM, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6:231–43

35] Boonstra A, Barrat FJ, Crain C, et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001;167:4974–80.

36] Davenport CB. Multiple sclerosis from the standpoint of geographic distribution and race. Arch Neurol Psychiatry 1922;8:51–

37] Acheson ED, Bachrach CA, Wright FM. Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation, and other variables. Acta Psychiatr Scand Suppl 1960;35:132–47

38] Simpson Jr S, Blizzard L, Otahal P, et al. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. J Neurol Neurosurg Psychiatry 2011;82:1132–41

39] McMichael AJ, Hall AJ. Does immunosuppressive ultraviolet radiation explain the latitude gradient for multiple sclerosis? Epidemiology 1997;8:642–5

40] Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol 2007;61:288–99.

41] Alonso A, Hernán MA. Temporal trends in the incidence of multiple sclerosis: a systematic review. Neurology 2008;71:129–35

42] Staples J, Ponsonby AL, Lim L. Low maternal exposure to vitamin D in pregnancy, month of birth and risk of multiple sclerosis in offspring: longitudinal analysis. BMJ 2010;340:c1640.

43] Willer CJ, Dyment DA, Sadovnick AD, et al. Timing of birth and risk of multiple sclerosis: population based study. BMJ 2005;330:120

44] Lucas R, Ponsonby AL, Dear K, et al. Sun exposure and vitamin D act independently in risk of central nervous system demyelination. Neurology 2011;76:540–8

45] Becklund BR, Severson KS, Vang SV, et al. UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. Proc Natl Acad Sci U S A 2010;107:6418–23.

46] Hart PH, Gorman S, Finlay-Jones JJ. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? Nat Rev Immunol 2011;11:584–96

47] Wang Y, Marling SJ, Zhu JG, et al. Development of experimental autoimmune encephalomyelitis (EAE) in mice requires vitamin D and the vitamin D receptor. Proc Natl Acad Sci U S A. 2012;109:8501–4

48] Islam T, Gauderman WJ, Cozen W, Mack TM. Childhood sun exposure infl uences risk of multiple sclerosis in monozygotic twins. Neurology 2007; **69:** 381–88..

49] Ramagopalan SV, Knight JC, Ebers GC. Multiple sclerosis and the major histocompatibility complex. Curr Opin Neurol 2009; **22:** 219–25.

50] Ferrari SL, Rizzoli R. Gene variants for osteoporosis and their pleiotropic eff ects in aging. Mol Aspects Med 2005; **26:** 145–67.

51] Thakkinstian A, D'Este C, Attia J. Haplotype analysis of VDR gene polymorphisms: a meta-analysis. Osteoporos Int 2004; **15:** 729–34.

52] Alberto Ascherio, Kassandra L Munger, K Claire Simon Vitamin D and multiple sclerosis Lancet Neurol 2010; 9: 599–612

53] Sarah Hewer a, Robyn Lucas b, Ingrid van der Mei c, Bruce V. Taylor, Vitamin D and multiple sclerosis. Journal of Clinical Neuro science 20 (2013) 634-641.

54] Chris h. polman, stephen c. reingold, brenda banwell ; Diagnostic criteria for multiple sclerosis: 2010 revisions to the mcdonald criteria ann neurol 2011;69:292–302

55] Michael f. holick, neil c. binkley, heike a. bischoff-ferrari, catherine m. gordon, david a. hanley, robert p. heaney, m. hassan murad, and connie m. weaver. Evaluation , treatment, and prevention of vitamin d deficiency: an endocrine society clinical practice guideline journal of clinical endocrinology & metabolism, july 2011, 96(7): 1911–1930

56] McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–127

57] Lekha Pandit, Sreeram V Ramagopalan, Chaithra Malli, Anitha D'Cunha, Ramya Kunder and Rajesh Shetty, Association of vitamin D and multiple sclerosis in India

Mult Scler. 2013 Oct;19(12):1592-6

58] Radhakrishnan Suresh Kumar, Sajid Syed, Anandakuttan Anand Kumar, K. N. Subha Kumari, K. Sajitha. Serum Vitamin D Levels in Indian Patients with Multiple Sclerosis Ind J Clin Biochem (July-Sept 2013) 28(3):255–258

59] J Smolders, P Menheere, A Kessels, J Damoiseaux and R Hupperts Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis *Mult Scler* 2008; 14; 1220

60] Barnes MS1, Bonham MP, Robson PJ, Strain JJ, Lowe-Strong AS, Eaton-Evans J, Ginty F, Wallace JM. Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 concentrations in male and female multiple sclerosis patients and control volunteers. Mult Scler. 2007 Jun;13(5):670-2. Epub 2007 Feb 16

61] Mavila Veetil et al Vitamin D status among the doctors in a tertiary referral centre in Kerala *Endocrine Abstracts* (2015) **37** EP228

62] John H. Lee, James H. O'Keefe, David Bell, Donald D. Hensrud, Michael F. Holick. Vitamin D Deficiency. An Important, Common, and Easily Treatable Cardiovascular Risk Factor Journal of the American College of Cardiology, Vol. 52, No. 24, 2008

63] Aksnes L, Aarskog D. Plasma concentrations of vitamin D metabolites in puberty: effect of sexual maturation and implications for growth. J Clin Endocrinol Metab 1982; 55: 94 101.

64] Kumar R, Cohen WR, Silva P, Epstein FH. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. J Clin Invest 1979; 63: 342 44

65] JJ Kragt1, BM van Amerongen2, J Killestein1, CD Dijkstra2, BMJ Uitdehaag Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women Multiple Sclerosis 2009; 15: 9–15

66] Spach KM, Hayes CE. Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. J Immunol 2005; 175: 4119 26.

67] I.A.F. van der Mei, A.-L. Ponsonby, T. Dwyer,L. Blizzard,B.V. Taylor,T. Kilpatrick,H. Butzkueven. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. J Neurol (2007) 254:581–590

68] J.M. Gelfand, Cree, J. McElroy, J. Oksenberg, R. Green,E.M. Mowry, Vitamin D in African Americans with multiple sclerosis. Neurology 2011;76:1824–1830

69] Lilyana Amezcua • Rachel H. Chung • David V. Conti • Annette M. Langer-Gould Vitamin D levels in Hispanics with multiple sclerosis. J Neurol (2012) 259:2565–2570

70] Ellen M. Mowry, Emmanuelle Waubant, Charles E. McCulloch, Darin T. Okuda, Vitamin D status predicts new brain MRI activity in multiple sclerosis.

Ann Neurol. 2012 August ; 72(2): 234–240

71] Masoud Nikanfar1, Ali Akbar Taheri-Aghdam1, Maria Yazdani1, Sheida Shaafi Serum 25(OH) Vitamin D levels is not associated with disability in multiple sclerosis patients: A case-control study. Iran J Neurol 2015; 14(1): 17-21

72] Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 1982;1:74–6.

73] Harris SS. Vitamin D and African Americans. J Nutr 2006;136:1126–1129

74] Steve Simpson, Bruce Taylor, Leigh Blizzard, Anne-Louise Ponsonby

Higher 25-hydroxyvitamin D Is Associated with Lower Relapse Risk in Multiple Sclerosis ANN NEUROL 2010;68:193–203

75] Tessel F. Runia, Wim C.J. Hop, Yolanda B. de Rijke, Dragan Buljevac, Rogier Q. Hintzen. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis Neurology® 2012;79:261–266

76] Ellen M. Mowry, Emmanuelle Waubant, Charles E. McCulloch, Darin T. Okuda, Vitamin D status predicts new brain MRI activity in multiple sclerosis, Ann Neurol. 2012 August ; 72(2): 234–240

77] Murat Yildiza, Barbara Tettenborna, Norman Putzkib Vitamin D levels in Swiss multiple sclerosis patients Swiss Med Wkly. 2011;141:

78] Ranking of states and union territories by literacy rate: 2011 Census of India Report (2013)

ANNEXURE

PROFORMA

1. Identification information

1.1) Serial number

1.2) Hospital number

1.3) Name

1.4) Residential address

1.5) Phone number

2. Details of Patient

2.1) Age _____ years (____ months)

2.2) Gender Male Female

2.3) Occupation

2.4) Education

2.5) Socioeconomic status

2.6) Date of birth

2.7) Smoking

2.8) Exposure to sunlight (Number of hours)

3 **Details of Multiple sclerosis**

3.1) Age at symptom onset

3.2) Age at diagnosis

3.3) Duration of disease

3.4) Number of relapses (Total)

3.5) Number of relapses prior to vitamin d detection

3.6) Number of relapses prior to vitamin D in last two year

3.7) Annualized relapse rate

3.8) the disease course can be characterized at present as

1 Relapsing-remitting (RR) MS

2 Secondary progressive (SP) MS

3. Primary progressive (PP) MS

4 Progressive relapsing (PR) MS:

3.9) EDSS (at the time of blood collection)

3.10) Disease severity

Mild

Moderate

Severe

3.11) Disease status (at the time of blood collection)

Relapse

Remission

3.12) Disease activity

4) Vitamin D

Vitamin D level

Vitamin D status

Sufficient

Insufficient

Deficient

TREATMENT DETAILS

On DMT

OFF DMT

Matched control

Age

Sex

Vitamin D

Vitamin D level

Vitamin D status

- Sufficient
- Insufficient
- Deficient