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Vitamin D status and antibody levels to common viruses in pediatric-onset multiple sclerosis

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Abstract

Background—The relative contribution and interaction of risk factors for multiple sclerosis (MS) have not been evaluated.

Objective—To determine if vitamin D status is associated with antibody levels to common viruses in pediatric-onset MS or clinically isolated syndrome (CIS) patients and controls.

Methods—We assessed if vitamin D status was associated with viral antibody levels to Epstein-Barr virus, cytomegalovirus (CMV), and herpes simplex virus(HSV) -1 or -2 in subjects who demonstrated evidence of remote infection with these viruses and whether these associations differed depending on disease status.

Results—In 140 subjects, vitamin D status was weakly associated with antibody levels to CMV but not to the other viruses. However, there were some interactions between vitamin D status and disease state. Among those with vitamin D sufficiency (≥ 30 ng/mL), MS/CIS patients had higher antibody levels to Epstein-Barr nuclear antigen-1 than controls. Vitamin D sufficiency was associated with higher CMV antibody levels in MS/CIS subjects but lower CMV antibody levels in controls. Higher vitamin D levels appeared to be associated with higher titers to HSV-2 in MS/CIS patients but not controls.

Conclusion—Vitamin D status may be differentially associated with antibody levels to common childhood viruses among seropositive subjects.

Keywords

multiple sclerosis; vitamin D; Epstein-Barr virus infections; cytomegalovirus; herpes simplex; risk factors

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INTRODUCTION

There is evidence that vitamin D insufficiency, infection with Epstein-Barr virus (EBV), cigarette smoking, and *HLA-DRB1*1501/1503* are risk factors for developing MS, and many of these factors may also influence the course of the disease.[1-5] The relative contributions of these factors to the risk and course of MS, as well as whether these factors interact to influence these outcomes, remain unclear.

The active form of vitamin D has potent immunomodulatory properties in both the innate and adaptive immune systems. It is plausible that vitamin D status may influence the immune response to viruses, and it has been hypothesized that in MS, low vitamin D levels predispose patients to EBV reactivation by allowing for more TH1 cell activation, a possible mechanism by which EBV increases MS risk.[6] Recent studies have demonstrated that vitamin D insufficiency is associated with a greater risk of common infections.[7] Further, low vitamin D levels in HIV are associated with a greater degree of disease progression, suggesting worse immunologic control.[8] No study has evaluated whether vitamin D status is associated with antibody response to viruses in patients with MS, but the question is important. For example, among patients with clinically isolated syndrome (CIS), higher titers to Epstein-Barr nuclear antigen-1 predict conversion to clinically-definite MS and more T2 hyperintensities on brain magnetic resonance imaging.[9] If vitamin D status is associated with the antibody response to viruses known or proposed to be associated with MS pathogenesis, then widespread vitamin D supplementation would need to be considered more carefully.

In a cohort of children with MS or CIS and controls, we sought to determine if vitamin D status is associated with antibody levels to common childhood viruses and whether these associations differ based on MS status.

PATIENTS AND METHODS

The study was approved by the institutional review boards of the University of California, San Francisco (UCSF) and State University of New York (SUNY) Stony Brook. The study was conducted at each institution's Regional Pediatric MS Center of Excellence. All patients are invited to participate in a longitudinal cohort study in which blood samples are collected at baseline and demographic and clinical data are captured at baseline and throughout the follow-up period. For this study, we included individuals enrolled in the cohort at these clinics between April, 2004 and February 2009. Patients included those with MS or CIS with symptom onset at age 18 or below; controls were those seen concurrently for whom CIS or MS was ruled out. Self-reported race was divided into two categories: white or [fully or partially] non-white. Self-reported ethnicity was coded as non-Hispanic or [fully or partially] Hispanic.

Levels of 25-hydroxyvitamin D_3 in baseline serum samples, hereafter referred to as "vitamin D levels," were assessed by batched chemiluminescent assay (ARUP Laboratories, Salt Lake City, UT) except for seven samples that were measured at a later time. A "sufficient" level was considered ≥ 30 ng/mL (conversion factor to SI units: 2.496). Two patients had levels that could not be resolved beyond a value of <4 ng/mL; for purposes of the analysis, they were assigned a value of 2 ng/mL. Batched EBV viral capsid antigen (VCA), cytomegalovirus (CMV), and herpes simplex virus (HSV)-1 and -2 assays (IgG) were performed blindly at Oklahoma Medical Research Foundation with normalized ELISAs and are presented as international standardized ratios with positive responses ≥ 1.1, negative responses <0.9 and equivocal responses between 0.91 and 1.09. All equivocal results were re-tested and repeated equivocal results were dropped from the analysis. Epstein-Barr

nuclear antigen-1 (EBNA-1) antibody status was measured using a standard ELISA assay and is presented in normalized optical densities, as described elsewhere.[10] We also performed genotyping to establish if patients carried the *HLA-DRB1*1501* or **1503* allele or not as described previously.[2]

Statistical analyses were performed using Stata 10.0 (StataCorp, College Station, TX). We used multivariate linear regression, with the outcome defined as the antibody level for each virus in patients who were seropositive for that virus. Predictors included in the models included vitamin D status (either as a continuous variable or as a binary variable [sufficient vs. insufficient]), age at the time of blood collection, *HLA-DRB1*1501/1503* status, race, and ethnicity. When the *p* value for the interaction term was <0.2, we explored for interactions between MS/CIS and vitamin D status. We also explored whether MS/CIS status or the day of the year on which the blood was drawn confounded the association of vitamin D status and viral antibody levels.

RESULTS

A total of 140 subjects (120 with MS or CIS and 20 controls; 76 from UCSF and 64 from Stony Brook) were included in the analyses after 19 (16 MS/CIS; 3 controls) were excluded because the status of vitamin D, *DRB1* or all viruses was not available. Patients with MS/CIS were slightly older than controls but were similar with respect to sex, race and ethnicity (Table 1). The median disease duration at blood collection (interquartile range) for the MS/CIS group was 1.2 years (0.1, 8.3). Control diagnoses included neuromyelitis optica, suspected neuromyelitis optica vs. sarcoidosis, acute disseminated encephalomyelitis, scleroderma en coup de sabre, Guillain-Barre syndrome, cerebral vasculitis, low-grade tumor, recurrent optic neuritis (with normal brain MRI and CSF), mitochondrial or metabolic disorders, suspected paraneoplastic syndromes, multiple cranial neuropathies, non-specific white matter lesions on brain magnetic resonance imaging, psychogenic disease, and unknown disorders. Patients were more likely than controls to be antibody-positive for VCA and EBNA-1, although the confidence intervals do not exclude an association of disease state with the other viruses (Table 1). The basic assumptions of the multivariate models appeared to be reasonably addressed, although there were some outliers (using DFBETA); sensitivity analyses excluding the outliers are presented with the full model results in Table 2.

Epstein-Barr Virus

Among those who were VCA positive, only Hispanic ethnicity was independently associated with higher VCA antibody levels (coefficient= 0.54, 95% CI [0.13, 0.96], p=0.011) in multivariate models in which vitamin D was a binary variable (Table 2). A similar association was detected when vitamin D was a continuous variable or when outliers were excluded. MS/CIS status did not appear to be a confounder when it was added to the models, nor did it interact with vitamin D status. Adding the day of the year on which the blood was drawn was not associated with a meaningful change in the estimates.

Among subjects who were positive for EBNA-1 antibodies, *DRB1*-positive status appeared to be associated with higher antibody levels to EBNA-1, although the association was attenuated when outliers were removed (Table 2). There was no meaningful difference when the blood draw date was added to the models. While there was no evidence of MS/CIS status as a negative confounder in the association between vitamin D status and EBNA-1 antibody levels, there was an interaction between the dichotomized vitamin D level and MS/CIS status (p=0.034 for interaction term). Among those with a sufficient vitamin D level, those with MS/CIS had higher EBNA-1 antibody levels (coefficient=0.49, 95% CI [0.02, 0.97], p=0.043) than controls. However, when vitamin D was insufficient, there appeared to be no

meaningful difference in EBNA-1 antibody levels between MS/CIS subjects and controls (p=0.52). This interaction was not apparent, however, in models in which vitamin D was a continuous variable.

Cytomegalovirus

In those who were positive for CMV, vitamin D sufficiency was associated with a higher CMV antibody level (coefficient 0.60, 95% CI [−0.06, 1.26], p=0.074). A similar association was discovered when vitamin D was modeled as a continuous variable; each 1 ng/mL greater vitamin D level was associated with a higher CMV antibody level (coefficient 0.03, 95% CI [0.00, 0.06], p=0.024) (Table 2). However, when outliers were removed, the association was not apparent. The effect of outliers could not be assessed when vitamin D was dichotomized because no patients with CMV antibodies had sufficient vitamin D levels after outliers were dropped.

In the more inclusive models, there was evidence of a strong interaction between vitamin D status and MS status (p=0.007 for interaction term when vitamin D levels dichotomized; p=0.035 when vitamin D was continuous). Among MS/CIS patients, vitamin D sufficiency was associated with a higher CMV antibody level (coefficient 1.04, 95% CI [0.36, 1.73], p=0.004), while sufficient vitamin D levels in controls were associated with lower CMV antibody levels (coefficient −1.10, 95% CI [−2.44, 0.25], p=0.11). When vitamin D was modeled as a continuous variable, greater vitamin D levels in MS patients were associated with higher antibody levels to CMV (coefficient 0.04, 95% CI [0.01, 0.07], p=0.004); however, the association of vitamin D levels and CMV antibody levels in controls appeared to be less meaningful than in the prior model (p=0.38). Regardless of how vitamin D was modeled, MS patients with lower/insufficient vitamin D had lower CMV antibody levels than controls. There was no evidence of MS confounding an association between vitamin D status and CMV antibody levels. Adding the day of the year on which the blood was drawn was not associated with a meaningful change in the estimates.

Herpes Simplex Virus-1 and -2

Only age appeared to be meaningfully associated with HSV-1 antibody levels. In multivariate models (with dichotomized vitamin D, *DRB1* status, race, and ethnicity), each one year older age at blood collection was associated with a lower HSV-1 antibody level (beta coefficient −0.16, 95% CI [−0.34, 0.02], p=0.085); the results were similar in models in which vitamin D was a continuous covariate (Table 2). There was no meaningful difference when the blood draw date was added to the models. There appeared to be a possible interaction between MS/CIS status and vitamin D level (p=0.18), but analyses stratified by MS/CIS status did not show meaningful differences. Interaction could not be assessed in models in which vitamin D was dichotomous because there were no controls positive for antibodies to HSV-1 who had sufficient vitamin D levels. MS/CIS status did not appear to be a confounder in the association between vitamin D levels and HSV-1 antibody levels.

Among HSV-2-positive patients, vitamin D levels were weakly associated with HSV-2 antibody levels when vitamin D was a continuous, but not a binary, variable (coefficient for 1 ng/mL greater vitamin D level= 0.03, 95% CI [0.00, 0.07], p=0.08); non-white race also tended to be associated with higher antibody levels (Table 2). Adding the day of the year on which the blood was drawn was not associated with a meaningful change in the estimates. MS/CIS status did not appear to confound the association of vitamin D and HSV-2 antibody levels, although in the models that had vitamin D as a continuous variable, there was evidence for a possible interaction (p=0.12 for interaction term). Among those with MS/CIS, each 1 ng/mL greater vitamin D level was associated with a higher HSV-2 antibody levels

(coefficient 0.05, 95% CI [0.01, 0.09], p=0.030), while vitamin D did not appear to be associated with the antibody levels in controls. Such an interaction was not apparent when vitamin D was treated as a binary covariate.

DISCUSSION

In this cross-sectional study, vitamin D levels in simple models did not appear to be meaningfully associated with antibody levels of common childhood viruses in individuals with evidence of remote infection. However, for some viruses, including EBV, CMV, and HSV-2, there appeared to be interactions of vitamin D status and MS/CIS status. While preliminary, our results suggest that among seropositive individuals, those with MS/CIS have differences in viral antibody responses compared to patients without the disease. Furthermore, for CMV and HSV-2, vitamin D sufficiency (or higher levels thereof) was paradoxically associated with higher antibody levels in MS/CIS patients, while such an association was absent or in the opposite direction in controls.

Vitamin D has several immunologic functions, many of which are associated with immune tolerance and reducing the pro-inflammatory state. While vitamin D insufficiency has been shown to increase the risk of developing MS and is associated with higher relapse rates in those with established disease,[11] a vitamin D response element was recently identified in the promoter region of *HLA-DRB1*15*, the gene believed to be critical to initiating the autoimmune response in MS, and 1, 25-dihydroxyvitamin D_3 increases the expression of the gene *in vitro*.[12] Higher antibody levels to EBNA-1 have been shown to correlate with inflammatory activity on brain MRI of patients with MS;[13] whether a similar association exists with other viruses or with the degree of viral suppression in general is unclear. However, that higher levels of vitamin D were associated with higher antibody levels to some of the viruses in this study suggests, along with the genetic study, that higher vitamin D levels could even be harmful in MS patients. As such, a randomized controlled trial of vitamin D supplementation is crucial.

The study has several limitations. First, it is cross-sectional, so a causal association can not be assumed. Second, the sample size was small, particularly because we restricted the analyses to those who were positive for antibodies to each virus. This led to limitations in model-checking for some models as well as to some imprecision in the measures of association. Further, the finding of a significant association in one group but not the other for some of the models (EBNA-1 and HSV-2) could be due to chance. Alternatively, the small sample size, particularly for controls, could have led us to miss true associations. The use of controls seen at the same center helps to reduce referral bias, but the fact that several of the controls had other diseases, many of them autoimmune, may have biased the results as well. Given putative overlap in autoimmune processes, however, one might have expected bias towards the null.

While our results are preliminary, the assessment of the relation between vitamin D status and viral titers is novel and deserves further attention. We plan to conduct a follow-up study in larger group of cases and particularly of healthy controls, which will allow for a more thorough investigation of the association of putative MS risk factors with viral antibody levels. Furthermore, we will follow the MS/CIS patients longitudinally to more carefully assess the possibility of a causal association between vitamin D and antibody levels to viruses.

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Table 1
Characteristics of patients and controls

	MS/CIS (n=120)	Controls (n=20)	*p* value
Age at blood collection	15.0 ± 3.5	13.8 ± 3.9	0.14
Female	76 (63)	12 (60)	0.78
Non-white race	30 (25)	3 (17)	0.42
Hispanic ethnicity	52 (44)	6 (33)	0.41
HLA-DRB1 positive	56 (47)	7 (35)	0.33
25-hydroxyvitamin D_3 level (ng/mL)	22.2 ± 9.7	22.7 ± 9.7	0.83
25-hydroxyvitamin D_3 level ≥ 30 ng/mL	22 (18)	4 (20)	0.86
VCA-positive	109 (91)	13 (65)	0.001
EBNA-1 positive	108 (91)	11 (55)	<0.0001
CMV-positive	32 (27)	8 (40)	0.22
HSV-1-positive	52 (44)	5 (25)	0.11
HSV-2-positive	27 (23)	6 (30)	0.46

All values presented as mean (± standard deviation) or number (%)

Missing values (number for whom missing): race (4); ethnicity (3); EBNA-1 status (1); HSV-1 status (2)

Table 2

Predictors of Viral Antibody Levels in Multivariate Models

	VCA		EBNA		CMV		HSV-1		HSV-2	
	Whole	**No outliers**	**Whole**	**No outliers**	**Whole**	**No outliers**	**Whole**	**No outliers**	**Whole**	**No outliers**
Model 1	**n=120**	**n=102**	**n=118**	**n=103**	**n=40**	**n=30**	**n=56**	**n=47**	**n=32**	**n=23**
Vitamin D	0.01 (−0.02, 0.03) p=0.63	0.00 (−0.02, 0.02) p=0.95	0.00 (−0.01, 0.00) p=0.25	0.00 (−0.004, 0.003) p=0.75	0.03 (0.00, 0.06) p=0.024	0.00 (−0.02, 0.03) p=0.71	−0.03 (−0.10, 0.04) p=0.37	−0.02 (−0.08, 0.03) p=0.36	0.03 (0.00, 0.07) p=0.08	0.04 (0.01, 0.07) p=0.015
Age (per year)	0.01 (−0.06, 0.07) p=0.83	−0.01, (−0.08, 0.05) p=0.64	0.00 (−0.01, 0.02) p=0.75	0.00 (−0.01, 0.01) p=0.76	0.03 (−0.05, 0.10) p=0.48	0.00 (−0.06, 0.05) p=0.87	−0.17 (−0.35, 0.01) p=0.071	−0.29 (−0.48, −0.10) p=0.003	0.05 (−0.05, 0.15) p=0.28	0.06 (−0.02, 0.13) p=0.13
DRB1 +	−0.02 (−0.44, 0.39) p=0.91	0.04 (−0.33, 0.40) p=0.84	0.08 (−0.01, 0.17) p=0.073	0.02 (−0.05, 0.08) p=0.62	0.10 (−0.45, 0.66) p=0.71	0.13 (−0.26, 0.51) p=0.50	0.15 (−1.19, 1.48) p=0.83	1.07 (−0.09, 2.22) p=0.071	−0.22 (−1.01, 0.57) p=0.57	−0.14 (−0.65, 0.38) p=0.58
Non-white	0.34 (−0.14, 0.81) p=0.16	0.14 (−0.29, 0.56) p=0.53	−0.03 (−0.13, 0.07) p=0.52	−0.05 (−0.13, 0.03) p=0.22	−0.22 (−0.99, 0.55) p=0.56	−0.37 (−0.97, 0.24) p=0.23	−0.19 (−2.23, 1.86) p=0.86	−2.08 (−3.94, −0.23) p=0.028	0.89 (−0.40, 2.18) p=0.17	1.26 (0.16, 2.36) p=0.027
Hispanic	0.58 (0.16, 1.01) p=0.008	0.50 (0.13, 0.87) p=0.009	−0.03 (−0.12, 0.06) p=0.50	−0.03 (−0.10, 0.04) p=0.35	0.45 (−0.23, 1.14) p=0.19	0.25 (−0.29, 0.80) p=0.35	0.80 (−1.01, 2.62) p=0.38	−0.06 (−1.65, 1.52) p=0.94	0.72 (−0.45, 1.88) p=0.22	0.80 (−0.01, 1.61) p=0.053
Model 2	**n=120**	**n=99**	**n=118**	**n=103**	**n=40**	**n=26**	**n=56**	**n=41**	**n=32**	**n=26**
Vitamin D	−0.17 (−0.70, 0.35) p=0.51	−0.29 (−0.83, 0.25) p=0.29	−0.05 (−0.16, 0.06) p=0.36	−0.03 (−0.11, 0.04) p=0.40	0.60 (−0.06, 1.26) p=0.074	(dropped)	−0.89 (−2.37, 0.58) p=0.23	−0.54 (−1.69, 0.61) p=0.35	0.45 (−0.45, 1.36) p=0.31	0.42 (−0.26, 1.10) p=0.21
Age (per year)	0.01 (−0.05, 0.08) p=0.75	−0.02 (−0.08, 0.04) p=0.59	0.00 (−0.01, 0.02) p=0.79	0.00 (−0.01, 0.01), p=0.55	0.03 (−0.04, 0.11) p=0.36	0.00 (−0.06, 0.07) p=0.87	−0.16 (−0.34, 0.02) p=0.085	−0.21 (−0.38, −0.05) p=0.013	0.05 (−0.05, 0.16) p=0.30	0.05 (−0.01, 0.12) p=0.12
DRB1 +	−0.02 (−0.44, 0.39) p=0.91	0.08 (−0.29, 0.44) p=0.67	0.08 (−0.01, 0.17) p=0.069	0.02 (−0.04, 0.08) p=0.57	0.06 (−0.51, 0.62) p=0.84	0.03 (−0.36, 0.43) p=0.87	0.18 (−1.15, 1.50), p=0.79	−0.21 (−1.33, 0.90), p=0.70	−0.21 (−1.03, 0.62) p=0.61	0.06 (−0.42, 0.54) p=0.80
Non-white	0.32 (−0.15, 0.79) p=0.18	0.12 (−0.30, 0.53) p=0.58	−0.02 (−0.12, 0.08) p=0.65	−0.02 (−0.10, 0.05) p=0.56	−0.40 (−1.2, 0.39) 0.31	−0.44 (−1.08, 0.21) p=0.17	0.01 (−2.01, 2.03) p=0.99	−0.10 (−2.27, 2.07) p=0.93	0.58 (−0.69, 1.84) p=0.36	0.58 (−0.22, 1.38) p=0.15
Hispanic	0.54 (0.13, 0.96) p=0.011	0.45 (0.10, 0.81), p=0.013	−0.02 (−0.11, 0.07) p=0.60	−0.02 (−0.09, 0.04) p=0.44	0.25 (−0.41, 0.90), p=0.45	0.15 (−0.36, 0.66) p=0.55	0.94 (−0.81, 2.70) p=0.29	1.09 (−0.80, 2.99) p=0.25	0.48 (−0.69, 1.66) p=0.41	0.35 (−0.42, 1.12) p=0.36

Model 1= continuous vitamin D; Model 2= binary vitamin D; no meaningful change if day of year of blood draw added; one or two subjects were dropped from each model due to missing covariates