



# Risk factors for multiple sclerosis and associations with anti-EBV antibody titers



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**Abstract** Multiple sclerosis (MS) is an inflammatory demyelination of the central nervous system. We investigated the prevalence of EBV seropositivity and other known risk factors for MS (age, smoking, low vitamin D) and their effect on anti-EBV antibody titers. We retrospectively studied 249 MS patients receiving care at the American University of Beirut Medical Center and 230 controls, during 2010–2014. EBV seropositivity was higher in MS patients compared to controls for both anti-VCA (99.5%; 97.2%) and anti-EBNA-1 (96.3%; 89.4%), and the titers were significantly higher in MS patients. MS patients had a significantly lower vitamin D level ( $15.5 \pm 8.3$  ng/ml) compared to controls ( $20.4 \pm 11.3$  ng/ml). The proportion of heavy smokers and overweight individuals was significantly higher in MS patients. Lebanese MS patients have risk factors similar to those in western countries. Older age and female gender were associated with a higher anti-VCA titer and male gender with a higher anti-EBNA-1.

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## 1. Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated disease characterized by inflammatory demyelination of the central

nervous system and progressive neurological deterioration. The cause of MS is yet to be elucidated but several genetic and environmental factors have been linked to increased risk of developing MS. The risk factors include female gender [1], exposure to Epstein–Barr virus (EBV) [2], low vitamin D (25-hydroxy vitamin D) status [3], low exposure to ultraviolet (UV) radiation [4], smoking [5–7], childhood and adolescent obesity [8–11], and having the HLA-DRB1\*15 allele [12–14].

EBV is a gamma-herpes virus that infects around 90% of the population and remains dormant within B-lymphocytes throughout life [15]. During acute EBV infection, anti-viral capsid antigen (VCA) antibodies appear first in almost all

*Abbreviations:* MS, multiple sclerosis; EBV, Epstein–Barr virus; VCA, viral capsid antigen; EBNA-1, Epstein–Barr nuclear antigen-1; EA, early antigen; 25(OH)D, 25-hydroxy vitamin D; EDSS, Expanded Disability Status Scale; BMI, body mass index.

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patients, followed by the gradual production of anti-EBV nuclear antigen-1 (EBNA-1) antibodies, which may not appear up to 6 months following the acute illness in some people [16]. More than 99% of patients diagnosed with MS are infected with EBV and have increasing titers of IgG antibodies against EBNA-1 before the onset of neurological symptoms [17–19]. Furthermore, studies on anti-VCA IgG antibody titers also found a correlation with MS, while anti-early antigen (EA) IgG antibody titers did not show a significant association with MS [20]. Yet, EBV reactivation has not been found to be correlated with increased MS relapse or progression [21]. A correlation between EBV antibody titers and the Expanded Disability Status Scale (EDSS) among MS patients is still a matter of debate [22–25].

Low vitamin D (25(OH)D) level is another risk factor for developing MS, and many studies have found an association between vitamin D deficiency and MS development, disability progression (or EDSS) [26–28], and increasing numbers of Gadolinium-enhancing lesions on MRI [29]. It was reported that vitamin D supplementation could decrease inflammatory cytokine production of CD8+ T cells in MS patients [30]. Even though there is a general consensus that EBV infection and low vitamin D level are independent risk factors [31], one study found that EBNA-1-specific T cells are susceptible to modulation by vitamin D treatment [32].

Interestingly, in addition to being a risk factor for MS, one study found smoking to be correlated with increased anti-EBNA-1 antibody titers in MS patients [33] while another found the same correlation to be age-dependent [34]. A positive correlation was reported between smoking and anti-VCA antibody titers in healthy females but not in males [35]. Yet, one case–control study failed to replicate this association between anti-EBNA-1 antibodies and smoking [36].

In Lebanon, the prevalence of hypovitaminosis 25(OH)D (<30 ng/ml) was reported to be around 78% in all age groups in spite of being a sunny country [37]. Smoking cigarettes and water-pipe is very prevalent among the Lebanese population, and it was found that 1 h of water-pipe smoking is equivalent to smoking 4–20 cigarettes [38]. On the other hand, the prevalence of EBV infection in the Lebanese population is unknown. Thus, given the high correlation between MS, EBV infection, vitamin D deficiency and smoking, and the increasing number of MS cases in Lebanon, we sought to determine the prevalence of EBV seropositivity in the general Lebanese population and in MS patients who are followed at the AUBMC MS center and whether it correlates with patient demographics or other MS risk factors.

## 2. Methodology

### 2.1. Study design

This was a retrospective case–control study on 249 MS patients receiving care at the Multiple Sclerosis Center at the American University of Beirut-Medical Center (AUBMC) between 2012 and 2014. The control group consisted of 230 normal individuals who participated in a study on vitamin D at AUBMC during the period of 2010–2012. Patient demographics were anonymized and serum samples were frozen at  $-80^{\circ}\text{C}$  until use. The study was approved by the American University of Beirut institutional review board.

### 2.2. Materials

Serum samples were tested in duplicates for anti-EBNA-1 and anti-VCA antibodies in the Clinical Chemistry laboratory at AUBMC. Abbott ARCHITECT System EBNA-1 IgG chemiluminescent immunoassay and Abbott ARCHITECT System VCA IgG chemiluminescent immunoassay were used according to the manufacturer's instructions. Seropositivity determination was according to the following values of Sample RLU/Cutoff RLU (S/CO): negative (<0.75), gray zone (0.75 to <1.00), and positive ( $\geq 1.00$ ). Serum 25(OH)D of controls was measured in the Endocrine core laboratory, at the American University of Beirut, via a protein binding assay using the DiaSorin RIA (Diasorin, Incstar, Sallugia, Italy). Serum samples of MS patients were tested for 25(OH)D level via Roche 25-OH Vitamin D kit, according to the manufacturer's instructions, in the Endocrine core laboratory at AUB-MC. Vitamin D level was divided into 4 categories: deficient (<10 ng/ml), insufficient (10–30 ng/ml), desirable (30–150 ng/ml), and toxic (>150 ng/ml).

### 2.3. Smoking history

Subjects were considered as smokers if they are currently smoking. Smoking status was also divided into none, less than 1 pack/day, 1–2 packs/day, and more than 3 packs/day. Subjects who smoke a water-pipe were also considered smokers where smoking 1 water-pipe/day was considered equivalent to smoking 1–2 packs/day.

### 2.4. Body mass index

The weight and height of MS patients were taken from the patient charts, which were filled at the time of blood withdrawal. The weight and height of controls were taken from the survey that was filled at the time of their recruitment and blood withdrawal. The BMI was then calculated according to the formula:  $\text{weight} / (\text{height})^2$ . The BMI values were then divided as: normal (<25), overweight (25–29.99), and obese ( $\geq 30$ ).

### 2.5. Statistical analyses

The Statistical Package for Social Sciences program (SPSS) version 20 was used for data entry and analyses. We carried out the descriptive statistics by providing the number and percent of each of the demographic variables for both cases and controls, whereas the continuous variables were summarized by providing the mean and standard deviation. Bivariate analyses were performed to identify significant demographics associated with MS in the Lebanese population, as well as to identify variables that are significantly associated with anti-EBNA-1 and anti-VCA IgG antibody titers. This was carried out by the chi-squared test and the independent sample *t*-test. Multivariate analyses were carried out to determine the variables affecting antibody titers and significant risk factors for developing MS, where odds ratio (OR) and 95% confidence interval (CI) were reported. EBV seronegative patients were excluded in these analyses.

### 3. Results

#### 3.1. Subject demographics

The study included 479 subjects, 249 of which were MS patients and 230 controls. MS patients were mostly relapsing remitting (78.7%), with a disease duration of  $5.9 \pm 7.1$  years and a mean EDSS of  $2.0 \pm 1.7$ , and 53.8% of patients were receiving disease-modifying therapy at the time of sampling and the majority (92/128) were receiving interferon (Table 1). Other medications were Fingolimod, Natalizumab, Glatiramer Acetate, and Methylprednisolone. Only 30.6% of our population, (MS and controls) had vitamin D levels in the desirable range. When the subjects who were taking vitamin D supplements were excluded, we found that only 11.9% of our population had a desirable level of vitamin D. The rate of EBV seropositivity was very high in our population (97.8% for anti-VCA and 93.7% for anti-EBNA-1).

Subjects were not matched for age (mean age was 46.4 and 37.8 years for controls and MS subjects, respectively) (Table 2) and thus this could be considered a pilot study with a convenience sample. On the other hand, cases and controls were matched for gender and the ratio of male to female was approximately 1:1.5 for both controls and MS subjects. There was no significant difference between the mean body mass index (BMI) of controls (26.5) and cases (25.9), but the proportion of overweight and obese individuals was higher in MS patients (58.3%) compared to controls (55%) ( $p = 0.05$ ). The ratio of smokers to non-smokers was around 1:1.5 for both MS and controls, but the proportion of heavy smokers was higher in MS patients where 30.6% of cases smoked 1 or more packs/day compared to 12.3% of controls ( $p < 0.0001$ ). Vitamin D levels were higher in MS ( $28.6 \pm 22.3$  ng/ml) than controls ( $23.3 \pm 13.6$  ng/ml) ( $p = 0.001$ ), but when we excluded subjects receiving vitamin D supplements, the relationship was reversed with a significantly lower vitamin D levels in MS subjects ( $15.5 \pm 8.3$  ng/ml) compared to controls ( $20.4 \pm 11.3$  ng/ml) ( $p < 0.0001$ ) and the proportion of MS patients with insufficient and deficient levels was higher (93.3%) compared to controls (83.2%). The proportion of EBV seropositivity was higher in MS patients (99.5% and 97.2%, respectively) compared to controls (96.3% and 89.4%, respectively) both for anti-VCA ( $p = 0.04$ ) and anti-EBNA-1 ( $p = 0.001$ ) and the anti-VCA

and anti-EBNA-1 titers were significantly higher in MS patients (52.4 and 19.8 S/CO, respectively) compared to controls (49.0 and 15.0 S/CO, respectively) ( $p = 0.05$  and  $p < 0.0001$ , respectively).

#### 3.2. Association between anti-VCA and anti-EBNA-1 and demographic variables

Table 3 shows the associations between the anti-VCA and anti-EBNA-1 antibody titers and different demographic variables among cases and controls. Age was found to be associated with anti-VCA antibody titers in the control group where titers were found to increase with age. Interestingly, this age association was not seen in MS patients where anti-VCA titers were flat and higher than controls at all age groups except those 14–19 years old. There was no association between age and anti-EBNA-1 antibody titers. Gender had no significant effect on antibody titers in either group. BMI, smoking status and the number of smoked packs per day had no significant association with antibody titers among both cases and controls. We found no association between vitamin D status and antibody titers in either cases or controls even after excluding subjects taking vitamin D supplements. However, MS patients with a deficient and insufficient level of vitamin D had a significantly higher mean level of anti-EBNA-1 antibody titers than controls, while those with a desirable level did not have a significantly different mean titer level from that of controls. Among MS patients, the disease duration had no association with the titers, while EDSS was negatively associated with anti-EBNA-1 antibody titers ( $p = 0.005$ ).

#### 3.3. Multivariate analysis of MS predictors

We examined the predictors of MS in our subjects. Among anti-VCA seropositive subjects, high anti-VCA antibody titer, younger age and higher number of smoked packs were significant predictors of MS (Table 4). Interestingly, low vitamin D level becomes a significant predictor of MS (OR: 0.43, 95% CI: 0.26–0.72, and  $p$ -value: 0.001) when subjects taking supplements were excluded (Table 4). Similarly, performing a multivariate analysis on subjects with an anti-EBNA-1 seropositive status yielded the same significant predictors, including low vitamin D level (OR: 0.36, 95% CI: 0.21–0.61, and  $p$ -value:  $<0.0001$ ) only when supplemented subjects were excluded (Table 4).

#### 3.4. Multivariate analysis of demographics affecting anti-EBV titer

We further assessed the effect of demographic variables and MS risk factors on anti-EBNA-1 and anti-VCA antibody titers, using a multivariate analysis. Among anti-VCA positive individuals, age ( $p < 0.0001$ ), female gender ( $p = 0.05$ ) and having MS ( $p = 0.003$ ) positively correlated with a higher anti-VCA titer (Table 5). Among anti-EBNA-1 positive individuals, only male gender ( $p = 0.009$ ) and having MS ( $p < 0.0001$ ) positively correlated with a higher anti-EBNA-1 titer (Table 5). These results were not affected when subjects taking vitamin D supplements were excluded (data not shown). Treatment with interferons had no effect on anti-VCA or anti-EBNA-1 titers.

**Table 1** Descriptive analysis of the 249 MS subjects.

Variables		Number (%)
Family history of multiple sclerosis	No	207 (83.8%)
	Yes	40 (16.2%)
Type of multiple sclerosis	Relapsing–remitting	196 (78.7%)
	Non-relapsing–remitting	53 (21.3%)
On medication	No	110 (46.2%)
	Yes	128 (53.8%)
Interferon treatment		92 (36.9%)
Disease duration in years	Mean ( $\pm$ sd)	5.9 ( $\pm$ 7.1)
Duration of medication in months	Mean ( $\pm$ sd)	13.8 ( $\pm$ 29.3)
EDSS	Mean ( $\pm$ sd)	2.0 ( $\pm$ 1.7)

**Table 2** Comparison of all the clinical and socio-demographic variables in both control and MS groups.

Variables		Control	Multiple sclerosis	p-Value	
		Number (%)	Number (%)		
Total sample		n = 230	n = 249		
Age	Mean ( $\pm$ sd)	46.4 ( $\pm$ 12.5)	37.8 ( $\pm$ 12.1)	<0.0001	
	14–19 years	1 (0.4%)	7 (2.8%)	<0.0001	
	20–29 years	18 (7.9%)	63 (25.3%)		
	30–39 years	53 (23.1%)	74 (29.7%)		
	40–49 years	59 (25.8%)	56 (22.5%)		
	50–59 years	59 (25.8%)	39 (15.7%)		
	60–69 years	39 (17.0%)	10 (4.0%)		
Gender	Male	84 (36.7%)	101 (40.6%)	0.38	
	Female	145 (63.3%)	148 (59.4%)		
BMI	Mean ( $\pm$ sd)	26.5 ( $\pm$ 5.0)	25.9 ( $\pm$ 4.8)	0.19	
	<25	89 (44.9%)	98 (41.7%)	0.05	
	25–29.99	63 (31.8%)	99 (42.1%)		
Smoking	$\geq$ 30	46 (23.2%)	38 (16.2%)	0.86	
	No	128 (59.3%)	149 (60.1%)		
Smocking Packs	Yes	88 (40.7%)	99 (39.9%)	<0.0001	
	None	128 (60.7%)	149 (60.1%)		
	Less than 1 pack/day	57 (27.0%)	23 (9.3%)		
	1–2 packs/day	25 (11.8%)	69 (27.8%)		
Vitamin D Level	3 or more packs/day	1 (0.5%)	7 (2.8%)	0.02	
	Mean ( $\pm$ sd)	23.3 ( $\pm$ 13.6)	28.6 ( $\pm$ 22.3)		
	Deficient	22 (11.2%)	42 (17.9%)		0.002
	Insufficient	125 (63.8%)	110 (46.8%)		
Excluding Supplemented Subjects (n = 288)	Desirable	49 (25.0%)	83 (35.3%)	<0.0001	
	Mean ( $\pm$ sd)	20.4 ( $\pm$ 11.3)	15.5 ( $\pm$ 8.3)		
	Deficient	20 (13.4%)	41 (30.1%)		<0.0001
	Insufficient	104 (69.8%)	86 (63.2%)		
Vitamin D Supplementation	Desirable	25 (16.8%)	9 (6.6%)	<0.0001	
	No	149 (77.6%)	139 (58.2%)		
Anti-VCA seropositivity	Yes	43 (22.4%)	100 (41.8%)	0.05	
	Mean ( $\pm$ sd)	49.0 ( $\pm$ 19.1)	52.4 ( $\pm$ 16.0)		
Anti-EBNA-1 seropositivity	Negative	8 (3.7%)	1 (0.5%)	0.04	
	Positive	207 (96.3%)	190 (99.5%)		
	Mean ( $\pm$ sd)	15.0 ( $\pm$ 6.7)	19.8 ( $\pm$ 5.1)	<0.0001	
	Negative	22 (10.6%)	7 (2.8%)	0.001	
	Positive	186 (89.4%)	242 (97.2%)		

#### 4. Discussion

This retrospective case–control study is the first to investigate the prevalence of EBV seropositivity in MS patients and the general population in Lebanon. Our results are in agreement with previous publications indicating an association between MS and exposure to EBV. EBV seropositivity was evaluated by testing for IgG antibodies against two viral antigens (EBNA-1 and VCA). Almost all MS subjects (190/191) showed reactivity to VCA antigen and a clear majority were also positive for anti-EBNA-1 antibodies. In spite of having a high rate of EBV seropositivity in the control group (higher than the 90% prevalence that is reported in the literature [39]), the association between EBV and MS remained significant. Interestingly, not all anti-VCA seropositive individuals were seropositive for anti-EBNA-1, suggesting that those people could either had a recent EBV infection or are not producing antibodies against

EBNA-1 or that there are false positives and negatives. In this study, we used a chemiluminescence assay that has been shown to have a higher sensitivity to anti-VCA and anti-EBNA-1, and higher specificity to anti-EBNA-1, than ELISA [40,41] while most of the reports relied on an EBNA-1 ELISA test to detect seropositivity. This could explain the higher prevalence observed in our study, although it is possible that this could reflect a true difference in prevalence.

Even though we found that anti-VCA seropositivity was significantly associated with having MS, the antibody titer was not significantly different between cases and controls, unlike what was previously reported [42,43]. On the other hand, the mean anti-EBNA-1 antibody titer was significantly higher in MS cases compared to controls. Interestingly, MS subjects with a desirable level of 25(OH)D tended to have a lower mean anti-EBNA-1 antibody titer similar to titers seen in controls. A larger study may allow us to tease out

**Table 3** Association between anti-EBNA-1, anti-VCA and all variables stratified by control group and MS group for patients with positive anti-VCA and anti-EBNA-1. \*: Association between all variables and anti-VCA and anti-EBNA-1; \*\*: Association between anti-VCA, anti-EBNA-1 and group control/MS split by groups in each variable.

Variables		Anti-VCA titer					Anti-EBNA-1 titer				
		Control group		MS group			Control group		MS group		
		Mean ( $\pm$ sd)/r	p value*	Mean ( $\pm$ sd)/r	p value*	p value**	Mean ( $\pm$ sd)/r	p value*	Mean ( $\pm$ sd)/r	p value*	p value**
Total sample		n = 207		n = 190		n = 397	n = 186		n = 242		n = 428
Age	Age	r = 0.27	<0.0001	r = 0.14	0.06		r = -0.008	0.92	r = -0.03	0.64	
	14–19 years	17.1 (-)	0.005	44.5 ( $\pm$ 15.0)	0.36	0.17	11.1 (-)	0.62	18.3 ( $\pm$ 4.5)	0.63	0.19
	20–29 years	40.3 ( $\pm$ 20.5)		50.2 ( $\pm$ 19.7)		0.13	12.5 ( $\pm$ 7.7)		20.2 ( $\pm$ 4.2)		0.002
	30–39 years	42.1 ( $\pm$ 20.5)		51.6 ( $\pm$ 13.3)		0.007	15.4 ( $\pm$ 6.2)		19.4 ( $\pm$ 5.8)		0.001
	40–49 years	50.4 ( $\pm$ 18.2)		53.7 ( $\pm$ 14.8)		0.33	15.8 ( $\pm$ 6.3)		20.4 ( $\pm$ 5.2)		<0.0001
	50–59 years	52.7 ( $\pm$ 18.1)		54.4 ( $\pm$ 17.1)		0.67	15.3 ( $\pm$ 6.7)		19.0 ( $\pm$ 5.1)		0.005
	60–69 years	54.0 ( $\pm$ 16.7)		61.9 ( $\pm$ 10.4)		0.24	14.2 ( $\pm$ 7.6)		20.4 ( $\pm$ 5.0)		0.006
Gender	Male	46.5 ( $\pm$ 19.9)	0.15	51.2 ( $\pm$ 16.6)	0.4	0.11	16.2 ( $\pm$ 6.2)	0.06	20.4 ( $\pm$ 4.8)	0.13	<0.0001
	Female	50.4 ( $\pm$ 18.7)		53.2 ( $\pm$ 15.6)		0.21	14.3 ( $\pm$ 6.9)		19.4 ( $\pm$ 5.2)		<0.0001
BMI	BMI	r = 0.04	0.62	r = 0.08	0.3		r = 0.01	0.88	r = 0.001	0.99	
	<25	47.2 ( $\pm$ 19.4)	0.56	53.1 ( $\pm$ 15.0)	0.56	0.03	14.7 ( $\pm$ 7.0)	0.7	19.4 ( $\pm$ 5.4)	0.71	<0.0001
	25–29.99	50.2 ( $\pm$ 19.4)		50.9 ( $\pm$ 17.2)		0.82	15.7 ( $\pm$ 6.3)		20.0 ( $\pm$ 4.6)		<0.0001
	$\geq$ 30	50.4 ( $\pm$ 17.3)		54.4 ( $\pm$ 16.5)		0.35	14.9 ( $\pm$ 6.9)		19.6 ( $\pm$ 5.7)		0.002
Smoking	No	47.3 ( $\pm$ 20.6)	0.16	51.3 ( $\pm$ 16.8)	0.26	0.11	14.5 ( $\pm$ 6.7)	0.21	20.0 ( $\pm$ 4.7)	0.43	<0.0001
	Yes	51.1 ( $\pm$ 17.0)		54.0 ( $\pm$ 14.9)		0.25	15.8 ( $\pm$ 6.9)		19.4 ( $\pm$ 5.6)		<0.0001
Number of packs	None	47.3 ( $\pm$ 20.6)	0.52	51.3 ( $\pm$ 16.8)	0.64	0.11	14.5 ( $\pm$ 6.7)	0.6	20.0 ( $\pm$ 4.7)	0.65	<0.0001
	Less than 1 pack/day	50.2 ( $\pm$ 17.4)		52.6 ( $\pm$ 11.9)		0.58	15.6 ( $\pm$ 7.1)		18.6 ( $\pm$ 7.0)		0.09
	1–2 packs/day	51.6 ( $\pm$ 17.9)		54.3 ( $\pm$ 16.2)		0.55	15.6 ( $\pm$ 7.1)		19.6 ( $\pm$ 5.2)		0.04
	3 or more packs/day	67.4 (-)		57.2 ( $\pm$ 13.7)		0.52	21.4 (-)		20.7 ( $\pm$ 5.3)		0.91
Vitamin D level	Mean	r = -0.01	0.87	r = -0.07	0.34		r = 0.04	0.64	r = 0.002	0.98	
	Deficient	53.2 ( $\pm$ 18.8)	0.42	53.3 ( $\pm$ 16.0)	0.34	0.98	15.3 ( $\pm$ 7.7)	0.75	19.0 ( $\pm$ 5.3)	0.63	0.04
	Insufficient	48.1 ( $\pm$ 19.5)		53.7 ( $\pm$ 15.5)		0.02	15.0 ( $\pm$ 6.8)		19.9 ( $\pm$ 5.3)		<0.0001
	Desirable	51.2 ( $\pm$ 17.8)		49.8 ( $\pm$ 16.8)		0.69	16.0 ( $\pm$ 6.3)		19.7 ( $\pm$ 4.7)		0.001
Vitamin D level of non-supplemented Subjects	Mean	r = -0.02	0.86	r = -0.04	0.71		r = 0.11	0.26	r = -0.05	0.54	
	Deficient	52.5 ( $\pm$ 19.2)	0.76	53.3 ( $\pm$ 16.0)	0.73	0.88	14.1 ( $\pm$ 7.4)	0.49	19.0 ( $\pm$ 5.3)	0.42	0.009
	Insufficient	49.2 ( $\pm$ 19.4)		54.4 ( $\pm$ 15.3)		0.06	15.3 ( $\pm$ 6.4)		19.7 ( $\pm$ 5.4)		<0.0001
	Desirable	50.8 ( $\pm$ 18.6)		50.0 ( $\pm$ 12.9)		0.91	16.7 ( $\pm$ 6.3)		17.3 ( $\pm$ 5.2)		0.8
EDSS	EDSS	-	-	r = 0.03	0.7		-	-	r = -0.20	0.002	
Disease duration	Disease duration	-	-	r = 0.06	0.45		-	-	r = -0.09	0.17	

**Table 4** Multivariate analysis for the predictors of MS among patients with positive anti-VCA or positive anti-EBNA-1.

Status	Predictors	All subjects		Subjects not Receiving Vitamin D Supplements	
		OR (95% CI)	p-Value	OR (95% CI)	p-Value
Positive anti-VCA	Anti-VCA titer	1.02 (1.01–1.03)	0.002	1.03 (1.01–1.04)	0.004
	Age	0.94 (0.92–0.95)	<0.0001	0.93 (0.90–0.95)	<0.0001
	Gender	0.83 (0.52–1.33)	0.44	0.84 (0.45–1.56)	0.57
	BMI	0.98 (0.94–1.03)	0.51	0.99 (0.93–1.06)	0.76
	Smocking packs	1.39 (1.07–1.81)	0.01	1.54 (1.09–2.17)	0.01
Positive anti-EBNA-1	Vitamin D level	1.36 (0.97–1.92)	0.08	0.43 (0.26–0.72)	0.001
	Anti-EBNA-1 titer	1.14 (1.10–1.19)	<0.0001	1.13 (1.08–1.19)	<0.0001
	Age	0.95 (0.93–0.96)	<0.0001	0.93 (0.91–0.96)	<0.0001
	Gender	1.23 (0.77–1.96)	0.40	1.30 (0.69–2.46)	0.41
	BMI	1.00 (0.95–1.05)	0.94	1.02 (0.96–1.08)	0.61
	Smocking packs	1.40 (1.07–1.83)	0.01	1.47 (1.02–2.11)	0.04
	Vitamin D level	1.28 (0.91–1.79)	0.16	0.36 (0.21–0.61)	<0.0001

this observation and longitudinal studies will be needed to determine a clear linkage between anti-EBV antibody titers and vitamin D replenishment.

An interesting observation was the increasing anti-VCA antibody titers with age in the control subjects, which was not observed in MS subjects. Longitudinal studies will be needed to document whether this cross-sectional observation is validated in individual patients.

In the multivariate analysis of risk factors for MS, we found that higher anti-VCA and anti-EBNA-1 titers, young age, high number of smoking packs, and low vitamin D level were associated with MS. These risk factors are similar to what was reported in the literature [3,42,44–46]. Gender did not show up as a risk factor, since cases and controls were matched for gender. We further performed another multivariate analysis to study the demographic variables affecting anti-EBV antibody titers and found that in addition to having MS, an older age and female gender were associated with a higher titer of anti-VCA while a higher anti-EBNA-1 titer was associated with male gender. Receiving Interferons did not significantly affect the mean antibody titer among MS patients.

Our study had several limitations, most important of which is that cases and controls were not age-matched. However since studies on MS risk factors and EBV sero-prevalence have not been previously done in Lebanon, this could be considered a pilot study with a convenience sample. Moreover, certain

demographic data were missing for some of the recruited subjects, including EBV seropositivity and titers, vitamin D level, smoking status, BMI, etc.

Another limitation was that the vitamin D level of controls and cases was measured via different methods, but this should not affect within-group analyses. Subjects were considered non-smokers based on their status at the time of study enrollment, so subjects who recently quit smoking may confound the results.

## 5. Conclusion

Our preliminary data indicate that MS environmental risk factors in Lebanon are similar to those reported in western countries, including young age, low vitamin D, heavy smoking, and EBV seropositivity with high titers. Further nation-wide sampling will be needed to confirm this observation. Longitudinal studies will be needed to better document the interaction of environmental and genetic risk factors.

## Disclosure

The authors of this manuscript declare no conflict of interest.

**Table 5** Multivariate analysis for the predictors of anti-VCA and anti-EBNA-1 titer among patients with positive anti-VCA and anti-EBNA-1 respectively.

Titer	Predictors	R square	Unstandardized beta	Standardized beta	95% CI	p-Value
Anti-VCA	Vitamin D level	0.06	−0.05	−0.04	−0.16: 0.06	0.40
	Smocking packs		1.60	0.08	−0.55: 3.75	0.15
	Age		0.28	0.21	0.13: 0.43	<0.0001
	Gender		3.75	0.10	−0.05: 7.54	0.05
	Control/MS		6.05	0.17	2.12: 9.98	0.003
Anti-EBNA-1	Vitamin D level	0.14	0.007	0.02	−0.02: 0.04	0.64
	Smocking packs		−0.06	−0.009	−0.74: 0.62	0.86
	Age		−0.009	−0.02	−0.06: 0.04	0.72
	Gender		−1.66	−0.13	−2.90: −0.42	0.009
	Control/MS		4.13	0.33	2.82: 5.43	<0.0001

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