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MAJOR ARTICLE

Solar Ultraviolet Radiation and Vitamin D Deficiency on Epstein-Barr Virus Reactivation: Observational and Genetic Evidence From a Nasopharyngeal Carcinoma-Endemic Population

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Background. We investigated the relationship of Epstein-Barr virus viral capsid antigen (EBV VCA-IgA) serostatus with ambient and personal ultraviolet radiation (UVR) and vitamin D exposure.

Methods. Using data from a multicenter case-control study, we included 1026 controls subjects in 2014–2017 in Hong Kong, China. Odds ratios (ORs) and 95% confidence intervals (CIs) of the association between UVR exposure and EBV VCA-IgA (seropositivity vs seronegativity) were calculated using unconditional logistic regression models adjusted for potential confounders.

Results. We observed a large increase in seropositivity of EBV VCA-IgA in association with duration of sunlight exposures at both 10 years before recruitment and age 19–30 years (adjusted OR = 3.59, 95% CI = 1.46–8.77; and adjusted OR = 2.44, 95% CI = 1.04–5.73 for ≥8 vs <2 hours/day; *P* for trend = .005 and .048, respectively). However, no association of EBV VCA-IgA serostatus with other indicators of UVR exposure was found. In addition, both circulating 25-hydroxyvitamin D (25OHD) and genetic predicted 25OHD were not associated with EBV VCA-IgA serostatus.

Conclusions. Our results suggest that personal UVR exposure may be associated with higher risk of EBV reactivation, but we did not find clear evidence of vitamin D exposure (observational or genetic), a molecular mediator of UVR exposure. Further prospective studies in other populations are needed to confirm this finding and to explore the underlying biological mechanisms. Information on photosensitizing agents, and serological markers of EBV, and biomarkers related to systemic immunity and inflammation should be collected and are also highly relevant in future studies.

Keywords. Epstein-Barr virus; genetic epidemiology; nasopharyngeal carcinoma; ultraviolet radiation; vitamin D.

Epstein-Barr virus (EBV) is the most common human virus, infecting and persisting latently in more than 90% of the adult population worldwide [1], but EBV only accounts for over 200 000 new cancer cases each year [2]. Although most infected individuals establish a life-long immunity to the virus and do not develop the associated illness, EBV can be reactivated and then cause clinical disease when the cellular immune response is compromised [3]. Immunosuppression is thought to contribute to EBV reactivation, and elevated risks in EBV reactivation have been observed among organ-transplantation recipients and human immunodeficiency virus patients [4], which may subsequently be associated with higher risks in EBV-related malignancies [5]. EBV reactivation also can be induced by deoxyribonucleic acid (DNA)-damaging agents [6, 7].

Solar ultraviolet radiation (UVR), an omnipresent nonionizing radiation, can damage DNA and induce immunosuppression [8], but the underlying mechanisms between UVR and EBV-related malignancies remain unclear. Higher risks in EBV-related diseases such as nasopharyngeal carcinoma (NPC) were found in individuals with higher exposure to solar UVR [9], whereas UVR was associated with lower risks of multiple sclerosis [10] and lymphomas [11, 12], suggesting that UVR-induced EBV reactivation may play a dual etiological role in human health. However, the precise role of UVR exposure in EBV reactivation is unknown, particularly for an ultimate biomarker of UVR with potential immunomodulatory and anti-inflammatory effects—vitamin

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D. Vitamin D, a surrogate of sunlight exposure, has traditionally been viewed as a contributor for the UVR-induced immunomodulation and anti-inflammation [13]. Few studies have assessed the association between vitamin D and EBV reactivation [14–16]. A cross-sectional study in 71 plasma samples of EBV-seropositive young adults in the United Kingdom showed no correlation of circulating 25-hydroxyvitamin D_3 $(25OHD)_3$ for EBV load or anti-EBNA-1 titers [14]. In a vitamin D supplementation study on 37 healthy Antarcticians, participants with higher serum 25OHD were more likely to have less EBV in saliva [15]. A randomized controlled trial in 53 patients with relapsing-remitting multiple sclerosis showed high-dose vitamin D_3 supplementation (14 000 IU/day; n = 30) reduced anti-EBNA-1 antibody levels [16]. These studies did not include other races/ethnicities (especially Chinese) and areas with higher UV levels due to latitude, both being strong modifying factors of vitamin D exposure. Moreover, these studies did not control for potential confounders, including smoking, occupation, socioeconomic status, dietary vitamin D intake, and ambient or personal UVR exposure.

We examined the associations of (1) EBV viral capsid antigen (VCA-IgA) serostatus with ambient and personal UVR and (2) vitamin D exposure using data from 1026 hospital-based non-NPC patients recruited in a multicenter NPC case-control study in 2014–2017 in Hong Kong, China where UV levels are high due to latitude while vitamin D deficiency is common [17] and NPC is endemic [18]. This is the first report that includes a comprehensive list of UVR exposure indicators (integrating both personal behavior and ambient UVR) [19], and it is the largest study to examine the associations between vitamin D exposure and EBV VCA-IgA serostatus using both serum vitamin D and a refined measure of vitamin D exposure (genetically instrumented based on single-nucleotide polymorphism (SNP) that relates to vitamin D synthesis and/or catabolism) [20–22].

MATERIALS AND METHODS

Patient Consent Statement

Informed consent was obtained from all individual subjects included in the study.

Study Approval

The Institutional Review Board of the HKU/Hospital Authority HK West Cluster (UW 11-192), the HK East Cluster Research Ethnics Committee (HKEC-2012-043), the Research Ethics Committee of the Hospital Authority Kowloon Central/Kowloon East (KC/KE-13-0115/ER-2), the Research Ethics Committee of the Kowloon West Cluster (KW/EX-13-073(63-11)), and the NTW Cluster Clinical and Research Ethics Committee (NTWC/CREC/1239-13) approved the study.

Study Subjects

Subject recruitment of the multicenter NPC case-control study was conducted from March 2014 to September 2017 in 5 major regional hospitals (Queen Mary Hospital, Pamela Youde Nethersole Eastern Hospital, Queen Elizabeth Hospital, Princess Margaret Hospital, and Tuen Mun Hospital) that treat up to 90% of all NPC new cases in Hong Kong.

Only non-NPC patients were included in the present analysis, whereas NPC cases were excluded. The non-NPC patients were selected from patients who attended the clinics or admitted to the hospitals with a wide range of medical diseases unrelated to NPC. These non-NPC patients were frequency-matched by age (5-year age groups) and sex to the NPC cases, and they were new patients or referrals of a new health complaint in the past 12 months in specialist outpatient clinics or new inpatients admitted in the past 3 months in the same hospitals. We excluded those who had any possibly NPC-related symptoms such as hearing problems, epistaxis, or cranial nerve palsy. Following the AsiaLymph guideline of the US National Cancer Institute [23], we also specified that no more than 15% of controls had the same specific type of disease. A limited number of specific diagnoses were further excluded, based on a known or suspected relation with vitamin D exposure and immunological, infectious, and/or inflammatory etiology. In addition, because of the potential associations of EBV reactivation with sleep disturbances, fatigue, and fever [24], 13 non-NPC patients with these conditions were excluded in the present analysis. The disease list is shown in Supplementary Part I. Ten milliliters of peripheral blood were collected at the same date of recruitment (centrifuged at 3000 rpm at 4°C for 10 minutes), and then all samples were stored at −80°C before measurements of EBV VCA-IgA serostatus, circulating 25OHD concentration, and DNA extraction for genotyping.

Exposure

Ambient and Personal Ultraviolet Radiation

Exposure to ambient UVR was derived by linking the date of blood taken reported by 1026 subjects to the Hong Kong Observatory (HKO) by using the daily mean UV index. The HKO used a Yankee Environmental Systems broadband UVB-1 pyranometer for measuring the UV index [25]. The daily mean UV index for a given day was defined as the averages of all the 15-minute mean UV Index values between 7 AM and 6 PM in the day. Information on personal UVR exposure over 4 life periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment) was collected by a computer-assisted, self-administered questionnaire with satisfactory test-retest reliability [26], including duration of sunlight exposure (reliability coefficients ranged from 0.3 to 0.9), use of sunscreens (0.3–0.5), and hand skin tone (0.4–0.6).

Vitamin D

Circulating 25OHD. Serum level of 25OHD was measured using validated enzyme immunoassay (Abbott ARCHITECT i2000SR). The sensitivity was 4.75 nmol/L and the range was

0–400 nmol/L, and no sample had a concentration below or above these limits. The intra-assay coefficient of variation was 4.3–8.1% by repeating measurements of 50 samples, and the reliability coefficient was acceptable (<10%). Circulating 25OHD was classified into 3 a priori categories based on clinically relevant cut-off points for the main analysis: <37.5 (deficient), 37.5 < 75 (insufficient), and ≥75 (sufficient) nmol/L.

Genetic Predicted 25OHD. Genomic DNA for genetic analysis was extracted from buffy coat using the ReliaPrep Blood gDNA Miniprep System (Promega, Madison, WI) extraction kits according to the manufacturer's instructions. Common genetic variants have been identified in the recent genome-wide association studies of circulating 25OHD level, and 8 variants that passed a genome-wide association threshold ($P < 5 \times 10^{-8}$) and had been replicated were selected [27–29]. These genetic instruments locate in or near 4 25OHD-related genes: 7-dehydrocholesterol reductase (DHCR7), cytochrome P450 family 2, subfamily R, polypeptide 1 (CYP2R1), group-specific component (GC), and cytochrome P450, family 24, polypeptide 1 (CYP24A1). The metabolic pathways of vitamin D have been shown (Supplementary Figure 1), including rs7977926, rs12785878, rs3829251 and rs11234027 (DHCR7), rs12794714 (CYP2R1), rs4588 and rs1155563 (GC), and rs6013897 (CYP24A1). All SNPs chosen had a minor allele frequency of ≥5%. Genotyping of these 8 SNPs was performed at the Centre for PanorOmic Sciences, The University of Hong Kong using the iPLEX assay on the MassARRAY System (Sequenom, San Diego, CA). The rs7944926 was excluded due to the deviation from Hardy-Weinberg equilibrium ($P < .05$) (Supplementary Table 1). Because the pairs of rs3829251 and rs11234027 (DHCR7) and rs4588 and rs1155563 (GC) were in linkage disequilibrium (D' > 0.80, the information from one can represent the other), only one of them (rs11234027 and rs4588) was selected as the candidate SNPs. Furthermore, rs12785878, rs11234027, and rs6013897 were excluded due to the weak instrument bias (F-statistic <10). Finally, 2 variants (rs12794714 and rs4588) were used in the present analysis to calculate a composite genetic score (linear continuous: 0–4) based on the summation method [22]. A higher score represented a proxy to greater lifelong status of vitamin D deficiency.

Outcome Assessment (Epstein-Barr Virus Viral Capsid Antigen Serostatus)

Antibody of EBV VCA-IgA was measured using a commercial kit (EUROIMMUN AG, Lübeck, Germany) based on the standard method of enzyme-linked immunosorbent assay in subjects who had agreed to provide blood. To minimize bias, the laboratory personnel was blinded to the disease status of the samples. A calibrator for calculation and a negative control and positive control for internal quality assessment were included on each plate. Results were evaluated semiquantitatively by calculating the ratio of the optical density (OD) value of the sample over the OD value of the calibrator, expressed as relative OD. According to the manufacturer's instruction, the serostatus of VCA-IgA was classified as seronegative (relative OD value, <1.2) or seropositive (relative OD value, ≥1.2).

Covariables

Information on demographic and lifestyle factors was collected by the questionnaire, including sex, age, socioeconomic status (ranged from −1 [lowest] to 13 [highest], calculated by the subject's and his/her father's and mother's education, housing type at age 10, personal income, and household income), smoking status, body mass index, family history of cancer, exposure to any occupational hazards, season when blood was taken, and salted fish consumption, dietary vitamin D intake, and total energy intake over 4 periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment).

Statistical Analysis

We examined the associations of EBV VCA-IgA (seropositivity vs seronegativity) with UVR exposure (daily mean UV index and duration of sunlight exposure, use of sunscreens, and hand skin tone) and vitamin D exposure (categorical serum 25OHD and composite genetic score) by calculating odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models adjusted for sex and 5-year age group, socioeconomic position score, smoking status (never and ever), consumption of salted fish (never and ever), exposure to any occupational hazards (never and ever), season of blood taking (winter and summer), body mass index (≥18.5–23.0, <18.5, ≥23.0–25.0, and ≥25.0), tertiles of dietary vitamin D intake (<12.4, ≥12.4–22.5, ≥22.5–40.7, and ≥40.7-<637 IU/day), and total energy intake over 4 life periods. To assess dose-response effect, a test for trend was examined for a model that included UV index, duration of sunlight exposure, hand skin tone, and serum 25OHD and composite genetic score as an ordinal variable. All statistical analyses were done with Stata version 15.0 (StataCorp LLC, College Station, TX), and all tests were 2-sided ($P < .05$ indicating statistical significance).

RESULTS

Ultraviolet Radiation Exposure and Epstein-Barr Virus Viral Capsid Antigen

Duration of sunlight exposures at both 10 years before recruitment and age 19–30 years were associated with higher seropositivity of EBV VCA-IgA with dose-response relationships (adjusted OR = 3.59, 95% CI = 1.46–8.77; and adjusted OR = 2.44, 95% CI = 1.04–5.73 for ≥8 vs <2 hours/day; P for trend = .005 and .048, respectively) (Table 1). No association of EBV VCA-IgA serostatus with duration of sunlight exposure at other periods (age 13–18 and 6–12 years), and with UV index, use of sunscreens and hand skin tone over different periods was found.

Table 1. Odds Ratio and 95% CI of EBV VCA-IgA (Seropositivity Versus Seronegativity) With Personal UVR Exposure in Hong Kong, China 2014–2017

	Number of EBV VCA-IgA Status		Age- and Sex-Adjusted Model			Multivariable Adjusted Model[a]		
Variable	Positive	Negative	OR	95% CI		OR	95% CI	
Daily Mean UV Index at the Date of Blood Taken, 0-6								
Low (0–2)	139	334	1 (ref.)			1 (ref.)		
Moderate (3–5)	118	301	0.95	0.71–1.28		0.99	0.69–1.44	
High (≥6)	23	46	1.19	0.69–2.04		1.61	0.83–3.13	
P for trend					.82			.96
10 Years Before Recruitment								
Duration of Sunlight Exposure, Hours/Day								
<2	112	350	1 (ref.)			1 (ref.)		
≥2–<5	88	277	1.00	0.73–1.38		1.13	0.77–1.65	
≥5–<8	32	51	2.03	1.24–3.33		1.74	0.96–3.18	
≥8	16	17	2.87	1.39–5.91		3.59	1.46–8.77	
P for trend					.001			.005
Use of Sunscreens								
Never	121	295	1 (ref.)			1 (ref.)		
Ever	126	402	0.78	0.58–1.05		0.86	0.60–1.22	
Hand Skin Tone, 1–3								
1 (light)	62	201	1 (ref.)			1 (ref.)		
2	150	364	1.31	0.93–1.84		1.41	0.94–2.13	
3 (dark)	36	133	0.85	0.53–1.36		0.67	0.38–1.20	
P for trend					.76			.39
Age 19–30 Years								
Duration of Sunlight Exposure, Hours/Day								
<2	100	317	1 (ref.)			1 (ref.)		
≥2–<5	98	292	1.08	0.78–1.50		1.16	0.79–1.69	
≥5–<8	32	61	1.71	1.05–2.79		1.35	0.74–2.45	
≥8	16	23	2.27	1.14–4.51		2.44	1.04–5.73	
P for trend					.007			.048
Use of Sunscreens								
Never	117	312	1 (ref.)			1 (ref.)		
Ever	129	383	0.92	0.69–1.24		1.06	0.74–1.50	
Hand Skin Tone, 1–3								
1 (light)	81	248	1 (ref.)			1 (ref.)		
2	123	320	1.17	0.85–1.63		1.27	0.87–1.87	
3 (dark)	44	129	1.03	0.67–1.57		0.96	0.57–1.61	
P for trend					.74			.85
Age 13–18 Years								
Duration of Sunlight Exposure, Hours/Day								
<2	83	224	1 (ref.)			1 (ref.)		
≥2–<5	125	371	0.93	0.67–1.29		1.05	0.71–1.54	
≥5–<8	25	79	0.84	0.50–1.42		1.11	0.61–2.01	
≥8	15	20	2.02	0.99–4.15		1.83	0.75–4.44	
P for trend					.49			.11
Use of Sunscreens								
Never	136	412	1 (ref.)			1 (ref.)		
Ever	112	284	1.24	0.92–1.66		1.32	0.93–1.86	
Hand Skin Tone, 1–3								
1 (light)	85	244	1 (ref.)			1 (ref.)		
2	114	290	1.16	0.83–1.61		1.17	0.80–1.71	
3 (dark)	49	162	0.85	0.57–1.28		0.81	0.50–1.31	
P for trend					.60			.55
Age 6–12 Years								
Duration of Sunlight Exposure, Hours/Day								
<2	82	272	1 (ref.)			1 (ref.)		
≥2–<5	129	333	1.31	0.95–1.81		1.44	0.99–2.09	
≥5–<8	32	72	1.45	0.89–2.35		1.49	0.84–2.64	

Table 1. Continued

Variable	Number of EBV VCA-IgA Status: Positive	Negative	Age- and Sex-Adjusted Model: OR	95% CI		Multivariable Adjusted Model[a]: OR	95% CI	
≥8	5	17	0.94	0.33–2.63		0.67	0.20–2.25	
P for trend					.17			.27
Use of Sunscreens								
Never	162	463	1 (ref.)			1 (ref.)		
Ever	86	223	1.09	0.80–1.48		1.16	0.81–1.66	
Hand Skin Tone, 1–3								
1 (light)	110	289	1 (ref.)			1 (ref.)		
2	89	242	0.99	0.71–1.37		1.02	0.70–1.48	
3 (dark)	45	151	0.77	0.52–1.15		0.62	0.38–1.01	
P for trend					.25			.09

Abbreviations: CI, confidence interval; EBV VCA-IgA, Epstein-Barr virus viral capsid antigen; OR, odds ratio; ref., reference; UVR, ultraviolet radiation.

[a]Multivariable adjusted model included all variables above, and sex, 5-year age group (frequency-matching in subject recruitment), and socioeconomic position score (ranged from −1 [lowest] to 13 [highest], and calculated by the subject's and his/her father's and mother's education, housing type at age 10, personal income, and household income), smoking status (ever/never), consumption of salted fish (ever/never), exposure to any occupational hazards (ever/never), season of blood draw (summer/winter), body mass index (<18.5/≥18.5–23.0/≥23.0–25.0/≥25.0), dietary vitamin D intake (<12.4/≥12.4–22.5/≥22.5–40.7/≥40.7–<637 IU/day), and total energy intake over 4 life periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment) as appropriate.

Vitamin D Exposure and Epstein-Barr Virus Viral Capsid Antigen

Both circulating 25OHD and genetic predicted 25OHD were not associated with EBV VCA-IgA serostatus (Table 2). A positive association (without dose-response relationship) between higher serum levels of 25OHD and EBV VCA-IgA seropositivity was found in the age- and sex-adjusted model (adjusted OR = 1.80, 95% CI = 1.04–3.14; *P* for trend = .06 for 75–<127.3 vs <37.5 nmol/L 25OHD), but this association appeared to be null after adjusting for potential confounders in Models 1 and 2.

DISCUSSION

Ultraviolet Radiation and Epstein-Barr Virus

This is the first report showing personal UVR exposure could be a potential inducer of EBV reactivation in an NPC-endemic region. We found strong evidence that longer duration of sunlight exposure was associated with EBV VCA-IgA seropositivity. These results remained robust with adjustment for multiple and relevant confounders. Although no study has examined such association, to some extent, our findings are consistent with previous studies of the positive association between UVR exposure

Table 2. Odds Ratio and 95% CI of EBV VCA-IgA Serostatus (Seropositivity vs Seronegativity) With Serum 25-Hydroxyvitamin D Concentration and Genetic Predicted 25-Hydroxyvitamin D Concentration in Hong Kong, China 2014–2017[a]

Variables (Number of EBV seropositivity vs Seronegativity)	Age- and Sex-Adjusted: OR	95% CI	Model 1[b]: OR	95% CI	Model 2[c]: OR	95% CI
Serum 25OHD, nmol/L						
<37.5 (61 vs 186)	1 (ref.)		1 (ref.)		1 (ref.)	
37.5-<75 (192 vs 500)	1.16	0.83–1.63	1.10	0.75–1.62	1.02	0.67–1.55
75-<127.3 (29 vs 47)	1.80	1.04–3.14	1.48	0.80–2.76	1.31	0.67–2.59
P for trend		.06		.27		.54
Composite Genetic Score Based on 2 Genetic Variants (rs1279471 and rs4588) Associated With Higher 25OHD (Approximately −3.4 nmol/L per 1 score/Allele Decreased; Ranged From 4 to 0)						
3–4 (31 vs 63)	1 (ref.)		1 (ref.)		1 (ref.)	
1–2 (180 vs 412)	0.75	0.44–1.27	0.85	0.46–1.55	0.82	0.43–1.54
0 (60 vs 160)	0.88	0.55–1.40	0.93	0.54–1.59	0.96	0.55–1.70
P for trend		.25		.57		.44

Abbreviations: 25OHD, 25-hydroxyvitamin D; CI, confidence interval; CYP2R1, cytochrome P450 family 2 subfamily R member 1; EBV VCA-IgA, Epstein-Barr virus viral capsid antigen; GC, group-specific component; OR, odds ratio; ref., reference; rs, RefSNPs.

[a]Adjusted for sex and 5-year age group (frequency-matching in subject recruitment).

[b]Model 1: adjusted additionally for putative nasopharyngeal carcinoma (NPC) risk factors (consumption of salted fish [ever/never], family history of cancer [no/yes, non-NPC/yes, NPC], exposure to any occupational hazards [ever/never], socioeconomic position score [ranged from −1 (lowest) to 13 (highest), and calculated by the subject's and his/her father's and mother's education, housing type at age 10, personal income, and household income], and smoking status [ever/never]).

[c]Model 2: Model 1 additionally adjusted for factors of vitamin D exposure (season of blood draw [summer/winter], daily mean UV index at the date of blood draw, and 10 years before recruitment duration of sun exposure [<2/≥2–<5/≥5–<8/≥8 hours/day], use of sunscreen [ever/never], and hand skin tone [1: light-3: dark], body mass index [<18.5/≥18.5–23.0/≥23.0–25.0/≥25.0], dietary vitamin D intake [<12.4/≥12.4–22.5/≥22.5–40.7/≥40.7-<637 IU/day], and total energy intake).

and herpes simplex virus (HSV) reactivation. Exposure to solar UVR was associated with higher risk of HSV reactivation [30, 31]. Evidence from a randomization controlled trial of the effect of sunscreen on UV-induced herpes labialis has suggested that UV light is a potent stimulus for inducing reactivation of herpes [32]. Solar UVR may be related to virus reactivation through immunosuppression [33]. Indeed, higher risks in EBV-related diseases have been consistently observed in patients with immunosuppressive diseases [4]. Furthermore, exposure to solar UV has recently been associated with higher circulating levels of cutaneous T cell-attracting chemokine (CTACK) in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (Z. M. M. et al 2020 using the PLCO data between 1991 and 2005; data not published) [34], in which CTACK has been linked to EBV reactivation [35].

Ultraviolet Radiation, Vitamin D, and Epstein-Barr Virus

Although the mechanism of UV-induced EBV reactivation is unknown, vitamin D, a circulating mediator reflective of recent UVR exposure, is thought to contribute through its immunomodulatory effect [36]. However, in the present analysis, we did not find any association between vitamin D exposure (either circulating 25OHD or genetic predicted 25OHD) and EBV VCA-IgA serostatus. The null association of our results is consistent with those of observational studies in patients with multiple sclerosis that examined circulating concentration of vitamin D and anti-EBV nuclear antigen (EBNA) complex IgG and EBNA-2 [37], EBV load, or anti-EBNA-1 IgG [38]. However, other studies showed inverse correlations between serum 25OHD and anti-EBNA-1 [39], and that vitamin D supplementation reduced anti-EBNA-1 titers [40]. Our study has provided additional robust evidence of the association between vitamin D and EBV with several strengths. First, because we additionally measured single nucleotide polymorphisms, which could be a proxy to represent lifelong status of vitamin D-deficiency, our study can limit potential selection bias and reverse causality that can be introduced if only circulating levels of vitamin D were analyzed [41]. Second, we adjusted for a comprehensive list of vitamin D-related factors and other potential confounders.

Strengths and Limitations

The present study had 2 additional strengths. First, we collected inclusive indicators of both ambient and personal UVR exposure over 4 life periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment), which showed satisfactory test-retest reliability [26], thus limiting recall errors (random and systematic). Second, the large sample size of the present analysis had 83.3% statistical power to detect a crude difference of 0.5 nmol/L or greater in 25OHD level between subjects with EBV VCA-IgA seropositivity and seronegativity [42]. However, this study had several limitations. First, we only used EBV VCA-IgA status as a proxy for EBV activation. Although there is no gold standard to evaluate EBV activation, using other serological markers to explore inducers of EBV activation is needed, including IgA antibody against latent membrane protein 1 and antibodies against EBNA-1, Zta, and EA. Second, the status of EBV reactivation and vitamin D may vary from time to time. In our study, VCA-IgA and 25OHD were only captured at 1 time point because we collected blood samples once per subject. The fluctuations of these markers, if any, cannot be documented and studied. Potential associations of EBV VCA-IgA serostatus with vitamin D exposure warrant further investigation in large prospective studies. Third, reverse causality of UVR exposure could be a concern, although we examined the associations over 4 life periods and similar results were observed. Fourth, although we had adjusted for the most relevant and potential confounders, residual confounding is still a possibility.

CONCLUSIONS

This is the first report with comprehensive examination of EBV reactivation with ambient and personal UVR, and vitamin D exposure, showing that longer duration of sunlight exposure per day was significantly associated with increased risk of EBV VCA-IgA seropositivity. However, vitamin D exposure (observational or genetic), a molecular mediator of UVR exposure, was not associated with EBV VCA-IgA. Further prospective studies in other populations are needed to confirm this finding and to explore the underlying biological mechanisms. Information on photosensitizing agents, and serological markers of EBV reactivation, and biomarkers related to systemic immunity and inflammation should be collected and are also highly relevant in future studies.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.
Supplementary Figure 1. Vitamin D pathway genetic polymorphisms.
Supplementary Table 1. Summary of variants used as genetically predicted 25OHD exposure in Hong Kong, China 2014–2017.
Supplementary Part I. Disease list of the non-NPC subjects for measuring Epstein-Barr virus (EBV) reactivation.

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Author contributions. Z.-M. M., Y.-H. C., and T.-H. L. designed the study; Z.-M. M. performed the statistical analysis and drafted the manuscript; Z.-M. M., J.-H. L., R. K.-C. N., D. L.-W. K., W.-T. N., A. W.-Y. N., A. W.-M. L., and M. L. L. collected data. All authors critically reviewed data for important intellectual content and contributed to final approval of the paper.

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References

1. Gourzones C, Busson P, Raab-Traub N. Epstein-Barr Virus and the Pathogenesis of Nasopharyngeal Carcinomas. In: Busson P, ed. *Nasopharyngeal Carcinoma: Keys for Translational Medicine and Biology*. New York: Springer-Verlag; **2013**: 42–60.
2. de Martel C, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol **2012**; 13:607–15.
3. Crawford DH, Rickinson A, Johannessen I. Cancer Virus: The Story of Epstein-Barr Virus. USA: Oxford University Press; **2014**.
4. Scheinberg P, Fischer SH, Li L, et al. Distinct EBV and CMV reactivation patterns following antibody-based immunosuppressive regimens in patients with severe aplastic anemia. Blood **2007**; 109:3219–24.
5. Grulich AE, Vajdic CM. The epidemiology of cancers in human immunodeficiency virus infection and after organ transplantation. Semin Oncol **2015**; 42:247–57.
6. Feng WH, Israel B, Raab-Traub N, et al. Chemotherapy induces lytic EBV replication and confers ganciclovir susceptibility to EBV-positive epithelial cell tumors. Cancer Res **2002**; 62:1920–6.
7. Westphal EM, Blackstock W, Feng W, et al. Activation of lytic Epstein-Barr virus (EBV) infection by radiation and sodium butyrate in vitro and in vivo: a potential method for treating EBV-positive malignancies. Cancer Res **2000**; 60:5781–8.
8. Bernard JJ, Gallo RL, Krutmann J. Photoimmunology: how ultraviolet radiation affects the immune system. Nat Rev Immunol **2019**; 19:688–701.
9. Chen W, Clements M, Rahman B, et al. Relationship between cancer mortality/incidence and ambient ultraviolet B irradiance in China. Cancer Causes Control **2010**; 21:1701–9.
10. Gallagher LG, Ilango S, Wundes A, et al. Lifetime exposure to ultraviolet radiation and the risk of multiple sclerosis in the US radiologic technologists cohort study. Mult Scler J **2019**; 25:1162–9.
11. Cahoon EK, Pfeiffer RM, Wheeler DC, et al. Relationship between ambient ultraviolet radiation and non-Hodgkin lymphoma subtypes: a U.S. population-based study of racial and ethnic groups. Int J Cancer **2015**; 136:E432–41.
12. Bowen EM, Pfeiffer RM, Linet MS, et al. Relationship between ambient ultraviolet radiation and Hodgkin lymphoma subtypes in the United States. Br J Cancer **2016**; 114:826–31.
13. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clinic Proc **2013**; 88:720–55.
14. Ramien C, Pachnio A, Sisay S, et al. Hypovitaminosis-D and EBV: no interdependence between two MS risk factors in a healthy young UK autumn cohort. Mult Scler **2014**; 20:751–3.
15. Zwart SR, Mehta SK, Ploutz-Snyder R, et al. Response to vitamin D supplementation during Antarctic winter is related to BMI, and supplementation can mitigate Epstein-Barr virus reactivation. J Nutr **2011**; 141:692–7.
16. Rolf L, Muris AH, Mathias A, et al. Exploring the effect of vitamin D3 supplementation on the anti-EBV antibody response in relapsing-remitting multiple sclerosis. Mult Scler **2018**; 24:1280–7.
17. Xu C, Perera RA, Chan YH, et al. Determinants of serum 25-hydroxyvitamin D in Hong Kong. Br J Nutr **2015**; 114:144–51.
18. Tang LL, Chen WQ, Xue WQ, et al. Global trends in incidence and mortality of nasopharyngeal carcinoma. Cancer Lett **2016**; 374:22–30.
19. Dadvand P, Basagaña X, Barrera-Gómez J, et al. Measurement errors in the assessment of exposure to solar ultraviolet radiation and its impact on risk estimates in epidemiological studies. Photochem Photobiol Sci **2011**; 10:1161–8.
20. Afzal S, Brøndum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. BMJ **2014**; 349:g6330.
21. Mokry LE, Ross S, Ahmad OS, et al. Vitamin D and risk of multiple sclerosis: a Mendelian randomization study. PLoS Med **2015**; 12:e1001866.
22. Smith JA, Ware EB, Middha P, et al. Current applications of genetic risk scores to cardiovascular outcomes and subclinical phenotypes. Curr Epidemiol Rep **2015**; 2:180–90.
23. Friesen MC, Lan Q, Ge C, et al. Evaluation of automatically assigned job-specific interview modules. Ann Occup Hyg **2016**; 60:885–99.
24. Glaser R, Padgett DA, Litsky ML, et al. Stress-associated changes in the steady-state expression of latent Epstein-Barr virus: implications for chronic fatigue syndrome and cancer. Brain Behav Immun **2005**; 19:91–103.
25. Dichter B, Beaubien A, Beaubien D. Development and characterization of a new solar ultraviolet-B irradiance detector. J Atmos Ocean Technol **1993**; 10:337–44.
26. Mai ZM, Lin JH, Chiang SC, et al. Test-retest reliability of a computer-assisted self-administered questionnaire on early life exposure in a nasopharyngeal carcinoma case-control study. Sci Rep **2018**; 8:7052.
27. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet **2010**; 376:180–8.
28. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet **2010**; 19:2739–45.
29. Cheung CL, Lau KS, Sham PC, et al. Genetic variant in vitamin D binding protein is associated with serum 25-hydroxyvitamin D and vitamin D insufficiency in southern Chinese. J Hum Genet **2013**; 58:749–51.
30. Ludema C, Cole SR, Poole C, et al. Association between unprotected ultraviolet radiation exposure and recurrence of ocular herpes simplex virus. Am J Epidemiol **2014**; 179:208–15.
31. Kawai K, VoPham T, Drucker A, et al. Ultraviolet radiation exposure and the risk of herpes zoster in three prospective cohort studies. Mayo Clin Proc **2020**; 95:283–92.
32. Rooney JF, Bryson Y, Mannix ML, et al. Prevention of ultraviolet-light-induced herpes labialis by sunscreen. Lancet **1991**; 338:1419–22.
33. Norval M. The effect of ultraviolet radiation on human viral infections. Photochem Photobiol **2006**; 82:1495–504.
34. Kitahara CM, Trabert B, Katki HA, et al. Body mass index, physical activity, and serum markers of inflammation, immunity, and insulin resistance. Cancer Epidemiol Biomarkers Prev **2014**; 23:2840–9.
35. Mao MJ, Xue N, Wang XP, et al. Chemokine CCL27 is a novel plasma biomarker for identification the nasopharyngeal carcinoma patients from the Epstein-Barr virus capsid antigen-specific IgA seropositive population. BMC Cancer **2018**; 18:9.
36. 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.
37. Munger K, Levin L, O'Reilly E, et al. Anti-Epstein–Barr virus antibodies as serological markers of multiple sclerosis: a prospective study among United States military personnel. Mult Scler J **2011**; 17:1185–93.
38. Mostafa A, Jalilvand S, Shoja Z, et al. Multiple sclerosis-associated retrovirus, Epstein-Barr virus, and vitamin D status in patients with relapsing remitting multiple sclerosis. J Med Virol **2017**; 89:1309–13.
39. Wergeland S, Myhr KM, Løken-Amsrud KI, et al. Vitamin D, HLA-DRB1 and Epstein-Barr virus antibody levels in a prospective cohort of multiple sclerosis patients. Eur J Neurol **2016**; 23:1064–70.
40. Røsjø E, Lossius A, Abdelmagid N, et al. Effect of high-dose vitamin D_3 supplementation on antibody responses against Epstein–Barr virus in relapsing-remitting multiple sclerosis. Mult Scler J **2017**; 23:395–402.
41. Kempker JA, Panwar B, Judd SE, et al. Plasma 25-hydroxyvitamin D and the longitudinal risk of sepsis in the REGARDS Cohort. Clin Infect Dis **2019**; 68:1926–31.
42. Mai ZM. The Relations of Nasopharyngeal Carcinoma With Vitamin D and Its Indicators: A Multicentre Case-Control Study in Hong Kong. Hong Kong: School of Public Health, The University of Hong Kong; **2019**.