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ORIGINAL ARTICLE

Association of lifestyle, psychological, and biological risk factors with multiple sclerosis: A case-control study

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ABSTRACT

Objectives: This study aimed to assess the prevalence and associations between lifestyle, psychological, and biological risk factors and the presence of multiple sclerosis (MS).

Patients and methods: A cross-sectional, observational, case-control study was conducted including 278 patients (105 males, 173 females; median age: 33 years; range, 16 to 61 years) with MS and 291 age-, sex-, and region-matched healthy controls (100 males, 191 females; median age: 33 years; range, 19 to 63 years) between January 1, 2013, and December 31, 2022. Multiple sclerosis diagnoses were based on national clinical guidelines. Risk factors assessed included smoking (active and passive), alcohol use (Alcohol Use Disorders Identification Test), physical activity (International Physical Activity Questionnaire-Short Form), vitamin D levels, Epstein-Barr virus (EBV) serostatus, body mass index, perceived stress (Perceived Stress Scale-10), anxiety (Generalized Anxiety Disorder-7), and depression (Patient Health Questionnaire-9). Data were collected using structured interviews and validated scales. Logistic regression (univariate and multivariate) was performed to estimate adjusted odds ratios (ORs) for MS risk, with additional interaction and sex-stratified analyses.

Results: Patients with MS had significantly higher rates of vitamin D deficiency (59.7% *vs.* 43.0%), EBV seropositivity (93.5% *vs.* 82.8%), physical inactivity (69.4% *vs.* 32.3%), high stress (41.4% *vs.* 29.2%), severe anxiety (27.0% *vs.* 14.8%), and severe depression (9.7% *vs.* 2.7%) compared to controls ($p<0.001$ for all). Multivariate regression identified the following independent protective factors: ≤9.9 pack-years of smoking (OR=0.30), no passive smoking before age 17 (OR=0.41), alcohol abstinence (OR=0.49), health-enhancing physical activity (OR=0.18) and minimal physical activity (OR=0.36), sufficient (OR=0.47) and insufficient (OR=0.51) vitamin D, EBV seronegativity (OR=0.35), moderate anxiety (OR=0.37), and mild depression (OR=0.25). A significant interaction between moderate stress and insufficient vitamin D levels was observed in females, with MS odds approximately 4.3 times higher in those exposed to both factors. Sex-stratified models indicated stronger associations with lifestyle factors in males and a greater contribution of psychological variables among females.

Conclusion: Multiple sclerosis susceptibility appears to be associated with multiple modifiable factors. Physical activity, vitamin D sufficiency, and EBV seronegativity showed strong negative associations with MS. Lifestyle-related exposures, such as alcohol use and smoking, were more strongly associated with MS in males, while psychological factors (stress, anxiety, and depression) demonstrated stronger associations in females. The observed interaction between moderate stress and insufficient vitamin D levels suggests a potential synergistic pattern that may warrant further investigation. These findings support the importance of a multifactorial perspective in MS research, emphasizing the need for sex-specific risk profiling. Longitudinal and mechanistic studies are needed to validate these associations and clarify potential causal pathways.

Keywords: Multiple sclerosis, sex, stress, risk factors, vitamin D.

Multiple sclerosis (MS) is a noncurable disorder of the central nervous system characterized by autoimmune responses, inflammation, and neurodegeneration.[1] The female-to-male ratio is approximately 3:1, and various genetic, environmental, lifestyle, psychological, and biological risk factors have been implicated in MS development. Among the most widely studied

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are immune-related genes, geographic latitude, ultraviolet radiation exposure (sunlight), female sex, race and ethnicity, Epstein-Barr virus (EBV) seropositivity, migration, low serum vitamin D levels, diet, obesity, tobacco use, alcohol abuse, stress, anxiety, depression, and others.[1-6] The coexistence and interaction of these risk factors often have a detrimental effect on disease progression. A thorough understanding and management of modifiable risk factors can play a crucial role in MS prevention and in halting disease progression.

This study aimed to assess the associations between various lifestyle, environmental, psychological, and biological risk factors and the risk of MS.

PATIENTS AND METHODS

This observational, cross-sectional, case-control study was conducted at the Neurology Center under the Ministry of Health of Azerbaijan. The study included MS patients identified from the MS registry of the Ministry of Health of Azerbaijan, covering the period from January 1, 2013, to December 31, 2022. The control group comprised healthy individuals without any neurological or autoimmune disorders. During the study period, 1,796 patients with MS were registered. Of these, 43 patients died during the follow-up period. From the remaining 1,753 individuals, a total of 278 patients (105 males, 173 females; median age: 33 years; range, 16 to 61 years) representing diverse regions of Azerbaijan were included in the study. The sample size was based on available participants meeting inclusion criteria with complete data. Participants with missing data on any of the variables included in a given analysis were excluded from that analysis using listwise deletion. Written informed consent was obtained from all participants. The study protocol was approved by the Neurology Centre of Ministry of Health's Ethics Committee (Date: 24.12.2012, No: 11/2012). The study was conducted in accordance with the principles of the Declaration of Helsinki.

All diagnostic procedures followed the national Clinical Protocol for the Diagnosis and Treatment of Multiple Sclerosis, as approved by the Ministry of Health of the Republic of Azerbaijan.[7] Inclusion criteria were as follows: a neurologist-confirmed diagnosis of MS, age ≥16 years, and ability to provide informed consent. Patients with varying degrees of disability, including those with high Expanded Disability Status Scale scores (e.g., 8-9), were included. For participants with significant physical or cognitive limitations, questionnaires and clinical scales were administered with the help of trained personnel or caregivers to ensure accurate and consistent data collection. Patients with comorbid psychiatric disorders (excluding depression and anxiety, which were separately assessed) were excluded.

A total of 291 individuals (100 males, 191 females; median age: 33 years; range, 19 to 63 years) were recruited as the control group. These participants were individuals accompanying patients at the neurology center but were not first-degree relatives. Controls were group-matched to MS patients based on age, sex, and region of residence to ensure comparability. During the matching process, sociodemographic characteristics were carefully considered. Exclusion criteria for the control group included any history of autoimmune, neurological, or psychiatric disorders.

All participants were assessed for a broad range of potential MS risk factors using structured questionnaires and validated instruments. For patients, risk factors were evaluated at the time of diagnosis or retrospectively and referred to exposures occurring prior to disease onset. For controls, it was assessed at the time of enrollment.

Among lifestyle factors, tobacco use was categorized as follows: current smokers (≥100 cigarettes in lifetime and currently smokes; daily or occasionally), former smokers (≥100 cigarettes in lifetime but does not currently smoke), and never smokers (<100 cigarettes in lifetime).[8] Active smoking exposure was quantified using the pack-year index, calculated as the number of cigarette packs smoked per day multiplied by the number of years the individual smoked. One pack was defined as 20 cigarettes. Smoking history was obtained via a structured questionnaire that included items on average daily cigarette consumption and total years of smoking. Based on cumulative exposure, participants were categorized as follows: "nonsmoker;" "≤9.9 pack year," "10-24.9 pack year," and "≥25 pack year."[9] Passive smoking was assessed via structured questions on exposure to secondhand smoke, categorized as "ever" *vs.* "never" exposed, with additional questions on exposure before age 17.

Alcohol consumption was assessed using the Alcohol Use Disorders Identification Test.[10]

Standard drinks were calculated for both groups, with one standard drink defined as containing 10 g of pure alcohol, as commonly referenced by the World Health Organization (WHO) in international studies.[11]

Physical activity assessment was evaluated using the International Physical Activity Questionnaire-Short Form (IPAQ-SF), which captures data on walking, moderate, and vigorous physical activities performed over the last seven days. Metabolic equivalent task (MET) values were assigned as follows: 3.3 METs for walking, 4.0 METs for moderate activity, and 8.0 METs for vigorous activity. The MET-min/week values were calculated by multiplying the MET value by the number of minutes and days each activity was performed. Based on the total MET-min/week and activity frequency, participants were categorized into three levels: insufficient physical activity, minimally active, and health-enhancing physical activity (HEPA), in accordance with the IPAQ-SF scoring protocol.[12,13]

Serum 25-hydroxyvitamin D [25(OH)D] levels were measured to determine vitamin D status, and classified as deficient (≤20 ng/mL), insufficient (21-29 ng/mL), and sufficient (≥30 ng/mL).[14] Blood samples for vitamin D analysis were collected during both summer and winter months for all participants. In patients with MS, samples were obtained during both relapse and remission phases, while in controls, sampling was conducted during the same seasonal periods to ensure comparability. Mean values across seasonal measurements were calculated and used for statistical analyses to account for intraindividual seasonal variation. Analyses were conducted using MAGLUMI 25-OH Vitamin D CLIA kits (chemiluminescence immunoassay; Snibe Diagnostics, Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China). Participants who were taking vitamin D supplements (patients or controls) were excluded from the study.

Participants' height (cm) and weight (kg) were measured using an electronic scale in the examination room to calculate the body mass index (BMI). The BMI was categorized based on a simplified version of the WHO classification: underweight (<18.4 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2).[15]

Serostatus of EBV was assessed via EBV viral capsid antigen (VCA) immunoglobulin (Ig) G antibody titers. Titers <4 AU/mL were considered negative; ≥4 AU/mL were considered positive. Testing was done using MAGLUMI EBV VCA IgG CLIA kits (Snibe Diagnostics, Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China).

Psychological factors (stress, anxiety, and depression) were retrospectively assessed for patients with MS using validated scales, with participants instructed to report symptoms experienced prior to diagnosis.

Perceived stress was measured using the Perceived Stress Scale-10. Scores were interpreted as low (0-13), moderate (14-26), and high (27-40) perceived stress.[16]

Anxiety was evaluated using the 7-item Generalized Anxiety Disorder (GAD-7) scale, which consists of seven items scored from 0 ("not at all") to 3 ("nearly every day"), resulting in a total score ranging from 0 to 21. Anxiety severity was categorized according to standard cutoff points as follows: 0-4 = minimal anxiety; 5-9 = mild anxiety; 10-14 = moderate anxiety; and 15-21 = severe anxiety.[17]

Depression was assessed using the Patient Health Questionnaire-9, which consists of nine items rated from 0 to 3, yielding a total score of 0 to 27. Scores were interpreted using established thresholds: 0-4 = minimal; 5-9 = mild; 10-14 = moderate; 15-19 = moderately severe; and 20-27 = severe depression.[18]

All questionnaires were administered in the Azerbaijani language. Where required, validated translations or versions with rigorous forward-backward translation procedures were used to ensure linguistic and cultural appropriateness.

Statistical analysis

Data were analyzed using IBM SPSS version 27.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were presented as medians with interquartile ranges (IQR), based on the results of normality tests (Shapiro-Wilk test). Categorical variables were presented as frequencies and percentages. Group comparisons were performed using the Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test with Phi or Cramér's V for categorical variables.[19]

Binary logistic regression was performed to assess associations between individual risk factors and MS status. For all categorical variables, the most severe or exposure-prone group (e.g., severe depression, high stress, EBV-positive, and vitamin D deficiency) was used as the reference category to allow consistent comparison across categories. Univariate logistic regression analyses were conducted first, and variables with p<0.05 were retained for multivariate modeling. Multivariate logistic regression was used to identify independent indicators of MS. Sex was included as a confounder due to its established role in MS risk, while other variables were not adjusted for based on preliminary analyses showing minimal confounding impact.

Education level was included in the univariate and main multivariate logistic regression models due to its significant group-level differences and theoretical relevance. However, it was excluded from the interaction and sex-stratified models to maintain model simplicity and preserve statistical power, as education was not a primary exposure of interest.

In logistic regression analyses, the dependent variable (MS status) was coded as "1" and the control as "0," so that odds ratios (Ors) reflected the odds of MS diagnosis associated with each risk factor relative to its reference category. A two-tailed p-value <0.05 was considered statistically significant.[19]

RESULTS

The MS and control groups showed no significant differences in age (median age: 33.0 years [IQR, 27.0-41.0 years] for both; p=0.249), sex distribution (62.2% *vs.* 65.6% female, p=0.398), or region of residence (64.4% *vs.* 61.9% urban, p=0.531; Table 1). Marital status (p=0.680), employment status (p=0.144), and monthly income (p=0.312) were also similar between groups. A significant difference was observed in education levels (p=0.021), with patients with MS having a higher proportion of higher education (27.3% *vs.* 17.9%) and controls having more secondary education (33.7% *vs.* 25.2%; Table 1).

TABLE 1
Baseline characteristics of patients with multiple sclerosis at the time of diagnosis *vs.* controls

	MS group (n=278)				Control group (n=291)				
	n	%	Median	IQR	n	%	Median	IQR	*p*
Age (year)			33.0	27.0-41.0			33.0	27.0-41.0	0.249*
Sex									
Female	173	62.2			191	65.6			0.398†
Male	105	37.8			100	34.4			
Residence									
Urban	179	64.4			180	61.9			0.531†
Rural	99	35.6			111	38.1			
Marital status									
Married	112	40.3			127	43.64			
Single	128	46.0			129	44.33			0.680†
Other	38	13.7			35	12.03			
Education level									
Primary	68	24.5			79	27.1			
Secondary	70	25.2			98	33.7			0.021†
Vocational	64	23.0			62	21.3			
Higher	76	27.3			52	17.9			
Employment status									
Employed	165	59.4			190	65.3			0.144†
Unemployed	113	40.6			101	34.7			
Income status									
No income	113	40.6			101	34.7			
Low (≤ minimum wage)	57	20.5			83	28.5			
Below average (1-2 × minimum wage)	47	16.9			52	17.9			0.312†
Average (2-3 × minimum wage)	26	9.4			25	8.6			
High (>3 × minimum wage)	21	7.6			19	6.5			
Prefer not to answer/unknown	14	5			11	3.8			

MS: Multiple sclerosis; IQR: Interquartile range; * Mann-Whitney U test; † Chi-square test.

TABLE 2
Comparison of main multiple sclerosis risk factors between patients at diagnosis and controls

	MS group (n=278)				Control group (n=291)				
Variables	n	%	Median	IQR	n	%	Median	IQR	*p*
Lifestyle Factors									
Smoking status									0.072†
Current	34	12.2			22	7.6			
Former	7	2.5			14	4.8			
Never	237	85.3			255	87.6			
Smoking status (binary)									0.407†
Ever	4	14.7			36	12.4			
Never	237	85.3			255	87.6			
Smoking duration (year)			14.0	9.0-22.5			10.0	5.0-19.5	0.125*
Smoking duration									0.232†
(grouped)	6	14.6			12	33.3			
≤5	5	12.2			6	16.7			
6-10	12	29.3			6	16.7			
11-15	5	12.2			5	13.9			
16-20	13	31.7			7	19.4			
≥21									
Smoking (pack year)			14.0	8.5-27.5			7.5	2.5-19.4	0.022*
Smoking (pack year 25)									0.445†
<25	30	73.2			29	80.6			
≥25	11	26.8			7	19.4			
Smoking (pack year 10)									0.005†
<10	12	29.3			22	61.1			
≥10	29	70.7			14	38.9			
Passive smoking									0.868†
No	190	68.3			197	67.7			
Yes	88	31.7			94	32.3			
Passive smoking before									0.037†
the age of 17	262	90.0			234	84.2			
No	29	10.0			44	15.8			
Yes									
Alcohol consumption									0.021†
Never consumed	204	73.4			239	82.1			
Former consumer	36	12.9			31	10.7			
Current consumer	38	13.7			21	7.2			
Physical activity (IPAQ SF)									
Total MET-min/week			172.00	0.00-1143.75			1415.00	306.00-1839.00	<0.001*
Inactive	193	69.4			94	32.3			
Minimally active	32	11.5			51	17.5			<0.001†
HEPA active	53	19.1			146	50.2			
Biological Factors									
BMI (kg/m²)			24.9	21.9-29.2			24.2	21.3-28.4	0.091*
BMI (kg/m²)									0.383†
Underweight	11	4.0			11	3.8			
Normal	131	47.1			158	54.3			
Overweight	78	28.1			72	24.7			
Obese	58	20.9			50	17.2			
EBV seropositivity									<0.001†
Positive	260	93.5			241	82.8			
Negative	18	6.5			50	17.2			

TABLE 2
Continued

	MS group (n=278)				Control group (n=291)				
Variables	n	%	Median	IQR	n	%	Median	IQR	*p*
Vitamin D average level (ng/mL) (seasonal average/ control group seasonal average)			18.39	13.73-26.05			22.19	17.44-30.42	<0.001*
Deficient	166	59.7			125	43.0			
Insufficient	59	21.2			89	30.6			<0.001†
Sufficient	53	19.1			77	26.5			
Psychological Factors									
Perceived stress, (PSS-10)			25	13-32			19	12-28	0.002*
Low stress	72	25.90			88	30.24			
Moderate stress	91	32.73			118	40.55			0.010†
High perceived stress	115	41.37			85	29.21			
Anxiety, GAD-7			6	3-15			5	2-11	0.002*
Minimal anxiety	107	38.49			136	46.74			
Mild anxiety	70	25.18			73	25.09			
Moderate anxiety	26	9.35			39	13.40			0.002†
Severe anxiety	75	26.98			43	14.78			
Depression, PHQ-9			3.0	2.0-13.0			3.0	2.0-5.0	<0.001*
Minimal depression	157	56.47			194	66.67			
Mild depression	35	12.59			55	18.90			
Moderate depression	29	10.43			18	6.19			
Moderately severe depression	30	10.79			16	5.50			<0.001†
Severe depression	27	9.71			8	2.75			

MS: Multiple sclerosis; IQR: Interquartile range; IPAQ SF: Physical Activity Questionnaire (Short Form); MET: Metabolic Equivalent Task; HEPA: Health-Enhancing Physical Activity; BMI: Body mass index; EBV: Epstein-Barr virus; PSS-10: Perceived Stress Scale; GAD-7: Generalized Anxiety Disorder 7-item; PHQ-9: Patient Health Questionnaire-9; * Mann-Whitney U test; † Chi-square test.

Smoking status at diagnosis showed no significant difference between patients with MS (12.2% current smokers) and controls (7.6%, p=0.072; Table 2). When analyzed as a binary variable (ever *vs.* never), no significant association was observed (14.7% *vs.* 12.6%, p=0.407; Table 2). Median smoking duration was 14.0 years (IQR 9.0-22.5 years) in patients with MS and 10.0 years (IQR, 5.0-19.5 years) in controls (p=0.125). Smoking duration by five-year categories was not significant (p=0.232). Cumulative smoking exposure was higher in patients with MS (median pack-years: 14.0 [IQR, 8.5-27.5] *vs.* 7.5 [IQR, 2.5-19.4], p=0.022; Table 2). No significant association was found for pack-years <25 *vs.* ≥25 (p=0.445), but ≥10 pack-years was significantly associated with MS status (70.7% *vs.* 38.9%, p=0.005; Table 2). Passive smoking exposure was similar between groups (31.7% *vs.* 32.3%, p=0.868), but passive smoking before age 17 was higher in patients with MS (15.8% *vs.* 10.0%, p=0.037), supported by continuity correction (p=0.049), likelihood ratio (p=0.036), and Fisher's exact test (p=0.044, two-sided).

Alcohol consumption differed significantly between groups (p=0.021), with current consumption more common in patients with MS (13.7% *vs.* 7.2%), and lifetime abstinence more frequent in controls (82.1% *vs.* 73.4%; Table 2). Physical activity was significantly lower in patients with MS (median: 172.0 MET-min/week [IQR, 0.0-1143.75 MET-min/week]) compared to controls (1415.0 MET-min/week [IQR, 306.0-1839.0 MET-min/week], p<0.001; Table 2). Patients with MS were more likely to be physically inactive (69.4% *vs.* 32.3%), while controls were more likely to have HEPA (50.2% *vs.* 19.1%, p<0.001). Body mass index showed no significant association with MS status (p=0.383; Table 2).

Epstein-Barr virus seropositivity was significantly higher in patients with MS (93.5% *vs.* 82.8%, p<0.001; Cramér's V=0.165). Vitamin D levels were lower in patients with MS (median: 18.39 ng/mL [IQR, 13.73-26.05 ng/mL]) compared to controls (22.19 ng/mL [IQR, 17.44-30.42 ng/mL], p<0.001; Table 2). Vitamin D deficiency was more common

TABLE 3
Univariate binary logistic regression analysis of risk factors associated with multiple sclerosis

			95% CI		
Variable (category)	Reference category	OR	Lower limit	Upper limit	*p*
Education level (secondary)	Primary	0.830	0.531	1.297	0.413
Education level (vocational)	Primary	1.199	0.745	1.931	0.455
Education level (higher)	Primary	1.698	1.052	2.742	0.030
Smoking status (non-smokers)	Current	0.601	0.342	1.058	0.078
Smoking status (former)	Current	0.324	0.113	0.928	0.036
Smoking, pack year (non-smokers)	>10 pack year	0.263	0.102	0.681	0.006
Smoking, pack year (≤9.9 pack year)	>10 pack year	0.449	0.231	0.870	0.018
Passive smoking exposure prior to MS (No)	Yes	1.030	0.724	1.466	0.868
Passive smoking exposure before the age of 17 (No)	Yes	0.589	0.357	0.971	0.038
Alcohol consumption (never drinkers)	Current drinkers	0.47	0.27	0.83	0.009
Alcohol consumption (former drinkers)	Current drinkers	0.64	0.31	1.32	0.226
MET-min/week (continuous)	N/A	0.999	0.999	0.999	<0.001
IPAQ SF (HEPA)	Insufficient activity	0.177	0.119	0.264	<0.001
IPAQ SF (minimal physical activity)	Insufficient activity	0.306	0.184	0.507	<0.001
Levels of vitamin D (sufficient)	Deficiency	0.518	0.341	0.789	0.002
Levels of vitamin D (insufficient)	Deficiency	0.499	0.334	0.747	<0.001
BMI (normal)	Obesity	0.715	0.459	1.114	0.138
BMI (underweight)	Obesity	0.862	0.344	2.157	0.751
BMI (overweight)	Obesity	0.934	0.569	1.533	0.787
EBV (seronegative)	Seropositive	0.334	0.189	0.588	<0.001
PSS-10 (low stress)	Severe stress	0.60	0.40	0.92	0.019
PSS-10 (moderate stress)	Severe stress	0.57	0.39	0.84	0.005
GAD-7 (minimal anxiety)	Severe anxiety	0.451	0.287	0.709	<0.001
GAD-7 (mild anxiety)	Severe anxiety	0.550	0.334	0.905	0.019
GAD-7 (moderate anxiety)	Severe anxiety	0.382	0.205	0.712	0.002
PHQ-9 (minimal depression)	Severe depression	0.240	0.106	0.543	<0.001
PHQ-9 (mild depression)	Severe depression	0.189	0.077	0.462	<0.001
PHQ-9 (moderate depression)	Severe depression	0.477	0.178	1.277	0.141
PHQ-9 (moderately severe depression)	Severe depression	0.556	0.205	1.503	0.247

OR: Odds ratio; CI: Confidence interval; MS: Multiple sclerosis; MET: Metabolic Equivalent Task; IPAQ SF: Physical Activity Questionnaire (Short Form); HEPA: Health-Enhancing Physical Activity; N/A: Not applicable; BMI: Body mass index; EBV: Epstein-Barr virus; PSS-10: Perceived Stress Scale; GAD-7: Generalized Anxiety Disorder 7-item; PHQ-9: Patient Health Questionnaire-9. Model significance based on likelihood ratio chi-square tests: Education level: $\chi^2(3)=9.730$, p<0.021; Smoking status: $\chi^2(2)=5.269$, p=0.072; Smoking (pack year): $\chi^2(2)=8.540$, p=0.014; Passive smoking exposure prior to MS: $\chi^2(1)=0.27$, p=0.868; Passive smoking exposure before age of 17: $\chi^2(1)=4.368$, p=0.037; Alcohol consumption: $\chi^2(2)=7.744$, p=0.021; MET-min/week: $\chi^2(1)=79.477$, p<0.001; Physical activity (IPAQ SF): $\chi^2(2)=81.707$, p<0.001; Vitamin D level: $\chi^2(2)=16.000$, p<0.001; BMI: $\chi^2(3)=3.060$, p=0.383; EBV seropositivity: $\chi^2(1)=15.490$, p<0.001; Perceived stress (PSS-10): $\chi^2(2)=9.296$, p=0.010; Anxiety: $\chi^2(3)=14.512$, p=0.002; Depression (PHQ-9): $\chi^2(4)=25.211$, p<0.001.

in patients with MS (59.7% *vs.* 43.0%), while sufficiency was less frequent (19.1% *vs.* 26.5%, p<0.001; Cramér's V=0.168).

Perceived stress was higher in patients with MS (median: 25 [IQR, 13-32]) than controls (19 [IQR, 12-28], p=0.002; Table 2). High stress was more prevalent in patients with MS (41.4% *vs.* 29.2%), while moderate stress was more common in controls (40.5% *vs.* 32.7%, p=0.010; Cramér's V=0.128). Anxiety was higher in patients with MS (median: 6 [IQR, 3-15] *vs.* 5 [IQR, 2-11], p=0.002), with severe anxiety more frequent in patients with MS (27.0% *vs.* 14.8%, p=0.002; Cramér's V=0.160). Depression was more severe in patients with MS (median: 3.0 [IQR, 2.0-13.0] *vs.* 3.0 [IQR, 2.0-5.0], p<0.001; Table 2), with severe depression more common in patients with MS (9.7% *vs.* 2.7%, p<0.001; Cramér's V=0.210).

Univariate logistic regression analyses identified several risk factors associated with MS status (Table 3). Education level was significantly associated with MS status. Individuals with higher education had greater odds of MS compared to those with primary/general education (OR=1.698, 95% confidence interval [CI]: 1.052-2.742, p=0.030). However, no significant associations were observed for secondary or vocational education levels (p=0.413 and p=0.455, respectively). Former smokers had lower odds of MS compared to current smokers (OR=0.324, 95% CI: 0.113-0.928, p=0.036; Table 3), while nonsmokers showed no significant difference (p=0.078). Nonsmokers and those with ≤9.9 pack-years had lower odds compared to >10 pack-years (p=0.006

TABLE 4
Multivariate binary logistic regression analysis of risk factors associated with multiple sclerosis

			95% CI		
Variable (category)	Reference category	OR	Lower limit	Upper limit	*p*
Education level					0.074
Education level (secondary)	Primary	0.972	0.580	1.629	0.916
Education level (vocational)	Primary	1.277	0.734	2.221	0.386
Education level (higher)	Primary	1.898	1.081	3.334	0.026
Smoking, pack year					0.029
Smoking, pack year (non-smokers)	>10 pack year	0.915	0.401	2.090	0.834
Smoking, pack year (≤9.9 pack year)	>10 pack year	0.271	0.087	0.848	0.025
Passive smoking exposure before the age of 17 (No)	Yes	0.391	0.212	0.723	0.003
Alcohol consumption					0.129
Alcohol consumption (never drinkers)	Current drinkers	0.544	0.268	1.101	0.090
Alcohol consumption (former drinkers)	Current drinkers	0.882	0.376	2.072	0.774
IPAQ SF					<0.001
IPAQ SF (HEPA)	Insufficient physical activity	0.177	0.112	0.281	<0.001
IPAQ SF (minimal physical activity)	Insufficient physical activity	0.369	0.210	0.648	<0.001
Levels of vitamin D					0.001
Levels of vitamin D (sufficient)	Deficiency	0.472	0.289	0.771	0.003
Levels of vitamin D (insufficient)	Deficiency	0.484	0.302	0.777	0.003
EBV (seronegative)	Seropositive	0.341	0.180	0.647	0.001
PSS-10					0.229
PSS-10 (low stress)	Severe stress	0.806	0.484	1.343	0.408
PSS-10 (moderate stress)	Severe stress	0.665	0.418	1.060	0.086
GAD-7					0.073
GAD-7 (minimal anxiety)	Severe anxiety	0.723	0.423	1.235	0.235
GAD-7 (mild anxiety)	Severe anxiety	0.616	0.342	1.111	0.107
GAD-7 (moderate anxiety)	Severe anxiety	0.389	0.189	0.800	0.010
PHQ-9					0.042
PHQ-9 (minimal depression)	Severe depression	0.430	0.164	1.128	0.086
PHQ-9 (mild depression)	Severe depression	0.245	0.086	0.699	0.009
PHQ-9 (moderate depression)	Severe depression	0.529	0.169	1.652	0.273
PHQ-9 (moderately severe depression)	Severe depression	0.634	0.198	2.035	0.444

OR: Odds ratio; CI: Confidence interval; IPAQ SF: Physical Activity Questionnaire (Short Form); HEPA: Health-Enhancing Physical Activity; EBV: Epstein-Barr virus; PSS-10: Perceived Stress Scale; GAD-7: Generalized Anxiety Disorder 7-item; PHQ-9: Patient Health Questionnaire-9. Overall significance (likelihood ratio χ^2 test) for each variable: Education level: $\chi^2(3)=9.730$, p<0.021; Vitamin D level: $\chi^2(2)=16.000$, p<0.001; EBV seropositivity: $\chi^2(1)=15.490$, p<0.001; Smoking (pack year): $\chi^2(2)=8.540$, p=0.014; Passive smoking <17 years: $\chi^2(1)=4.368$, p=0.037; Alcohol consumption: $\chi^2(2)=7.744$, p=0.021; Perceived stress (PSS-10): $\chi^2(2)=9.296$, p=0.010; Anxiety (GAD-7): $\chi^2(3)=14.512$, p=0.002; Depression (PHQ-9): $\chi^2(4)=25.211$, p<0.001; Physical activity (IPAQ SF): $\chi^2(2)=81.707$, p<0.001.

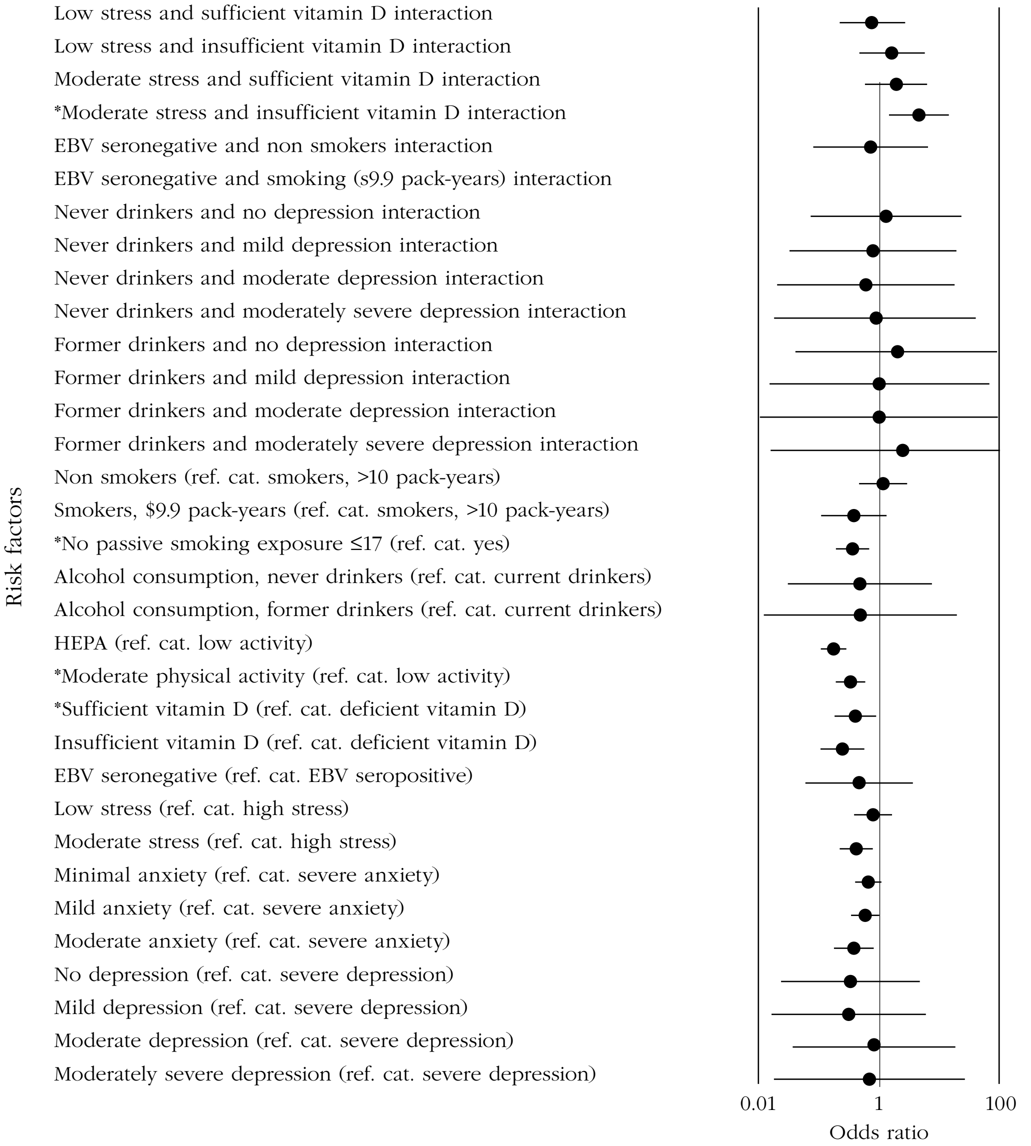

Figure 1. Multivariate logistic regression analysis of risk factors and interaction effects associated with multiple sclerosis.

EBV: Epstein-Barr virus; HEPA: Health-Enhancing Physical Activity, ref. cat. - reference category. Asterisks (*) indicate variables with statistically significant associations (p<0.05).

and p=0.018, respectively). No passive smoking before age 17 was associated with lower odds (OR=0.589, 95% CI: 0.357-0.971, p=0.038; Table 3). Never drinkers had lower odds compared to current drinkers (OR=0.47, 95% CI: 0.27-0.83, p=0.009). Higher physical activity levels (MET-min/week) were negatively associated with MS (p<0.001), with HEPA (OR=0.177, 95% CI: 0.119-0.264, p<0.001) and minimal activity (OR=0.306, 95% CI: 0.184-0.507, p<0.001) showing lower odds of MS compared to insufficient activity (Table 3). Sufficient and insufficient vitamin D levels had lower odds compared to deficiency (p=0.002 and p<0.001, respectively). Epstein-Barr virus seronegativity was associated with lower odds (OR=0.334, 95% CI: 0.189-0.588, p<0.001; Table 3). Low and moderate stress had lower odds compared to severe stress (p=0.019 and p=0.005, respectively). Lower anxiety levels were more frequently reported by controls than patients with MS, compared to those with severe anxiety (p<0.001 to p=0.019). Similarly, minimal and mild depression were more common among controls than those with severe depression (p<0.001; Table 3).

TABLE 5
Sex-stratified multivariate logistic regression results for multiple sclerosis risk factors

Variables	Sex	OR	95% CI Lower limit	95% CI Upper limit	*p*
Stress and vitamin D interaction	Male				0.520
	Female				0.186
Low stress and sufficient vitamin D interaction	Male	0.760	0.085-6.784		0.806
	Female	0.831	0.170-4.059		0.819
Low stress and insufficient vitamin D interaction	Male	2.191	0.237	20.269	0.490
	Female	1.819	0.368	8.993	0.463
Moderate stress and sufficient vitamin D interaction	Male	0.910	0.107	7.759	0.931
	Female	3.249	0.720	14.652	0.125
Moderate stress and insufficient vitamin D interaction	Male	6.452	0.638	65.228	0.114
	Female	4.279	1.057	17.317	0.042
Smoking, pack year	Male				0.343
	Female				0.008
Non smokers (reference category-smokers, >10 pack year)	Male	0.421	0.112	1.576	0.199
	Female	2.797	0.758	10.325	0.123
Smokers, ≤9.9 pack year (reference category-smokers, >10 pack year)	Male	0.277	0.044	1.767	0.175
	Female	0.241	0.034	1.727	0.157
No passive smoking exposure ≤17 (reference category-yes)	Male	0.243	0.093	0.632	0.004
	Female	0.556	0.216	1.433	0.225
Alcohol consumption	Male				0.008
	Female				0.966
Alcohol consumption, never drinkers (reference category-current drinkers)	Male	0.238	0.077	0.734	0.013
	Female	1.112	0.368	3.364	0.851
Alcohol consumption, former drinkers (reference category-current drinkers)	Male	0.801	0.214	2.996	0.741
	Female	0.995	0.247	4.002	0.994
Physical activity	Male				0.000
	Female				0.000
HEPA (reference category-inactivity)	Male	0.094	0.039	0.228	0.000
	Female	0.223	0.123	0.405	0.000
Minimal physical activity (reference category-inactivity)	Male	0.308	0.101	0.940	0.039
	Female	0.273	0.129	0.575	0.001
Vitamin D level	Male				0.065
	Female				0.022
Sufficient vitamin D (reference category-deficient vitamin D)	Male	0.360	0.078	1.664	0.191
	Female	0.350	0.122	1.008	0.052
Insufficient vitamin D (reference category-deficient vitamin D)	Male	0.136	0.025	0.730	0.020
	Female	0.256	0.087	0.756	0.014
EBV seronegative (reference category-EBV seropositive)	Male	0.305	0.096	0.962	0.043
	Female	0.332	0.142	0.778	0.011
Perceived stress	Male				0.898
	Female				0.002
Low stress (reference category-high stress)	Male	0.711	0.164	3.079	0.648
	Female	0.736	0.283	1.916	0.530

TABLE 5
Continued

Variables	Sex	OR	95% CI		*p*
			Lower limit	Upper limit	
Moderate stress (reference category-high stress)	Male	0.865	0.222	3.366	0.834
	Female	0.254	0.114	0.568	0.001
Anxiety	Male				0.661
	Female				0.060
Minimal anxiety (reference category-severe anxiety)	Male	0.879	0.322	2.404	0.802
	Female	0.509	0.254	1.020	0.057
Mild anxiety (reference category-severe anxiety)	Male	0.538	0.181	1.599	0.265
	Female	0.513	0.237	1.113	0.091
Moderate anxiety (reference category-severe anxiety)	Male	0.671	0.156	2.889	0.593
	Female	0.284	0.111	0.725	0.008
Depression	Male				0.794
	Female				0.013
Minimal depression (reference category-severe depression)	Male	0.221	0.020	2.430	0.217
	Female	0.455	0.143	1.449	0.183
Mild depression (reference category-severe depression)	Male	0.193	0.015	2.438	0.204
	Female	0.204	0.058	0.725	0.014
Moderate depression (reference category-severe depression)	Male	0.224	0.014	3.565	0.289
	Female	0.605	0.154	2.376	0.471
Moderately severe depression (reference category-severe depression)	Male	0.196	0.013	2.864	0.234
	Female	1.421	0.307	6.571	0.653

OR: Odds ratio; CI: Confidence interval; HEPA: Health-Enhancing Physical Activity; EBV: Epstein-Barr virus.

The multivariate model, adjusting for all covariates, was significant (chi-squared [χ^2] (22)=159.78, p<0.001; Nagelkerke R^2=0.326), with 71.2% classification accuracy (sensitivity=69.8%, specificity=72.5%; Hosmer-Lemeshow p=0.353). As demonstrated in Table 4, a higher educational level was independently associated with greater odds of MS (OR=1.898, 95% CI: 1.081-3.334, p=0.026). Factors independently associated with lower odds of MS in the multivariate model included ≤9.9 pack-years (OR=0.271, 95% CI: 0.087-0.848, p=0.025), no passive smoking before age 17 (OR=0.391, 95% CI: 0.212-0.723, p=0.003), HEPA (OR=0.177, 95% CI: 0.112-0.281, p<0.001), minimal physical activity (OR=0.369, 95% CI: 0.210-0.648, p<0.001), sufficient vitamin D (OR=0.472, 95% CI: 0.289-0.771, p=0.003), insufficient vitamin D (OR=0.484, 95% CI: 0.302-0.777, p=0.003), EBV seronegativity (OR=0.341, 95% CI: 0.180-0.647, p=0.001), moderate anxiety (OR=0.389, 95% CI: 0.189-0.800, p=0.010), and mild depression (OR=0.245, 95% CI: 0.086-0.699, p=0.009).

The model with interaction terms was significant (χ^2(33)=163.05, p<0.001; Nagelkerke R^2=0.332), with 71.7% classification accuracy (Hosmer-Lemeshow p=0.147). The interaction between moderate stress and insufficient vitamin D was significant (OR=4.279, 95% CI: 1.057-17.317, p=0.009). Other interactions (EBV-smoking, alcohol-depression) were not significant (p=0.951 and p=0.997, respectively; Figure 1).

Sex-stratified multivariate models showed good fit (males: Nagelkerke R^2=0.469, p<0.001; females: Nagelkerke R^2=0.354, p<0.001), with a classification accuracy of 76.1% for males and 71.2% for females. The moderate stress and insufficient vitamin D interaction was significant in females (OR=4.279, 95% CI: 1.057-17.317, p=0.042) but not in males (p=0.114; Table 5). No passive smoking before age 17 was significant in males (OR=0.243, 95% CI: 0.093-0.632, p=0.004) but not in females (p=0.225). Never drinking was more common among male controls than male patients with MS (OR=0.238, 95% CI: 0.077-0.734, p=0.013) but not in females (p=0.851). Health-enhancing physical activity was more common among controls than patients with MS in both sexes (males: OR=0.094, 95% CI: 0.039-0.228, p<0.001; females: OR=0.223, 95% CI: 0.123-0.405, p<0.001; Table 5). Minimal physical activity was observed more frequently in controls than patients with MS in females (OR=0.273, 95% CI: 0.129-0.575, p=0.001) and males (OR=0.308, 95% CI: 0.101-0.940, p=0.039). A negative association was observed between insufficient vitamin D levels and MS in both males (OR=0.136, 95% CI: 0.025-0.730, p=0.020) and females (OR=0.256, 95% CI: 0.087-0.756, p=0.014; Table 5). A negative association was also observed between EBV seronegativity and MS in both sexes (males: OR=0.305, 95% CI: 0.096-0.962, p=0.043; females: OR=0.332, 95% CI: 0.142-0.778, p=0.011). Moderate stress (OR=0.254, 95% CI: 0.114-0.568, p=0.001), moderate anxiety (OR=0.284, 95% CI: 0.111-0.725, p=0.008), and mild depression (OR=0.204, 95% CI: 0.058-0.725, p=0.014; Table 5) were reported less frequently by female patients with MS than female controls, but no such differences were observed in males.

DISCUSSION

This case-control study identified significant associations between MS and various lifestyle, psychological, and biological risk factors, with physical activity, vitamin D status, EBV seropositivity, smoking exposure, alcohol consumption, and psychological factors emerging as key predictors. The inclusion of interaction terms and sex-stratified analyses further elucidates the complex, multifactorial etiology of MS, highlighting both universal and sex-specific risk profiles.

The absence of significant differences in age, sex, and residence between patients with MS and controls confirms adequate baseline similarity for valid comparison of MS risk factors, consistent with rigorous case-control study designs.[20] A significant difference in educational attainment was observed, with patients with MS having a higher proportion of higher education (27.3% *vs.* 17.9% in controls). This aligns with a previous study from Australia reporting a higher prevalence of MS among individuals with higher education.[21] This association was further supported by univariate logistic regression analysis. This may reflect greater health-seeking behavior, access to diagnostic services, or a real association between educational level and MS risk. However, it also raises the need to consider education as a potential confounding factor in the analysis of risk factors, as it may influence exposure to other variables such as lifestyle or healthcare access. However, studies from Norway show inverse association between education levels and risk of MS.[22,23]

Smoking exposure, particularly cumulative pack-years (≥10) and early-life passive smoking, was associated with higher odds of MS. Current smokers had higher odds of MS compared to former and never smokers, although only the association with former smoking reached statistical significance in univariate analyses. A dose-response pattern was observed, with greater smoking exposure corresponding to higher MS odds, consistent with previous studies linking smoking to MS through proinflammatory mechanisms.[24] Additionally, Firat et al.[25] reported that smoking increases depression levels in MS patients, or conversely, that individuals with depression may be more likely to smoke. Passive smoking before age 17 was also associated with higher odds of MS, in line with earlier research reporting adolescent exposure to secondhand smoke as a potential contributor to MS susceptibility.[26,27] However, given the limited predictive capacity of the passive smoking model in our study, this finding should be interpreted with caution.

Alcohol consumption showed a negative association, with never drinkers demonstrating lower odds of MS compared to current drinkers. This finding raises important questions about the role of alcohol in MS risk. Although routine screening and intervention for alcohol use are not common in MS care,[28,29] existing evidence suggests that strict recommendations for complete abstinence may not be evidence-based and could even be counterproductive.[30] Therefore, our findings should be interpreted cautiously, considering potential confounders such as socioeconomic status, place of residence, or associated health behaviors. Further research is warranted to clarify whether moderate alcohol consumption might have a neutral or potentially protective role in MS development.

Physical activity showed a strong negative association with MS, with HEPA and minimally active individuals demonstrating lower odds of MS compared to those who were inactive. These findings are consistent with previous studies suggesting that higher levels of physical activity may be inversely related to MS risk.[31,32] Further investigation through longitudinal studies is warranted to establish causality and explore underlying mechanisms. Similarly, vitamin D sufficiency and insufficiency were associated with lower odds of MS compared to deficiency (by 53% and 49%, respectively), consistent with previous evidence suggesting a link between vitamin D deficiency and the presence of MS.[33,34] This inverse gradient likely reflects the immunomodulatory role of vitamin D in MS pathogenesis.

Epstein-Barr virus seropositivity was significantly more common among patients with MS (93.5%) compared to controls (82.8%), with seronegativity associated with 65% lower odds of MS. This finding is consistent with the well-documented association between EBV exposure and increased MS susceptibility, potentially mediated by mechanisms such as molecular mimicry or immune dysregulation.[35,36]

Although the distribution of patients by BMI in our study was quite similar to that reported by Akbay et al.[37] in Türkiye, the proportion of patients with obesity was higher in their study compared to ours (25.0% *vs.* 20.9%). As in our study, BMI was not significantly associated with MS status.[37] Psychological factors, including perceived stress, anxiety, and depression, were more prevalent and severe in patients with MS. Severe stress was more commonly reported by patients with MS in univariate analysis, while low and moderate stress were reported more frequently by controls. This pattern suggests an inverse gradient across stress levels. Psychological stressors appear to have a minor to modest impact on MS disease onset, inflammatory activity, and progression.[38] Furthermore, stressful life events have been associated with MS in prior studies, including the largest population-based case-control study to date.[39] Anxiety severity, as measured by the GAD-7 scale, showed an inverse association with MS, with lower anxiety levels more frequently observed in controls than patients with MS. Severe depression was more common in patients with MS (9.7%) than in controls (2.7%), while minimal and mild depression were more frequently reported by controls. Compared to severe depression, these lower levels of depression were associated with 76% and 81% lower odds of MS, respectively. These findings suggest that severe depressive symptoms are more prevalent and severe among individuals with MS, underscoring the need for mental health screening and support in this population.[40] Management of the psychosocial factors and social support mechanisms of patients may have an impact on their quality of life.[41] The attenuation of stress and moderately severe depression in the multivariate model suggests mediation or confounding by other factors, such as lifestyle or biological variables.

The multivariate model revealed a statistically significant interaction between moderate stress and insufficient vitamin D, with MS odds approximately 4.5 times higher overall and 4.3 times higher in females. While this interaction reached statistical significance in females, small cell sizes may have affected the stability of the estimates. Given the observational nature of the study and the potential limitations of interaction modeling, these findings should be considered exploratory and hypothesis-generating rather than confirmatory. This suggests a synergistic effect, where the combination of moderate stress and insufficient vitamin D heightens MS risk compared to severe stress and vitamin D deficiency. This finding conflicts with expected biological gradients, as severe stress with deficiency was hypothesized to confer higher risk. The vitamin D × stress interaction, while statistically significant for one combination, requires cautious interpretation due to potential overfitting, as indicated by model nonconvergence (maximum iterations reached). Further validation is recommended to clarify its biological plausibility. Nonsignificant interactions, such as EBV-smoking and alcohol-depression, were likely underpowered due to wide confidence intervals and small subgroup sizes, limiting their interpretability.[42]

However, Hedström et al.[42] have reported a significant interaction between current smoking and EBV seropositivity associated with the risk of MS. According to Taylor et al.,[43] moderate alcohol intake is associated with a lower risk of depression in patients with MS, suggesting that alcohol use may modulate psychological outcomes in MS, although causality and directionality remain uncertain.

Sex-stratified analyses highlighted distinct risk profiles. Health-enhancing physical activity and minimal physical activity, as well as EBV seronegativity, were negatively associated with MS in both sexes, while other lifestyle factors demonstrated sex-specific patterns. In males, no passive smoking before age 17 and never drinking were associated with 76% and 63% lower odds of MS, respectively. These findings suggest that lifestyle-related exposures, such as tobacco smoke and alcohol use, may have a stronger influence in males. In females, moderate stress, moderate anxiety, and mild depression were reported more frequently by controls than patients with MS, suggesting a stronger influence of psychological factors in this group. Vitamin D insufficiency showed a negative association with MS in both sexes, with a stronger effect in males. Sex differences were evident in the impact of risk factors, with females appearing more biologically vulnerable, as vitamin D insufficiency, elevated stress, and anxiety had stronger associations with MS risk. In contrast, the proportion of never drinkers and individuals with no reported passive smoking exposure before age 17 was higher among male controls than male patients with MS, with no significant differences observed in females. Epstein-Barr virus seronegativity was associated with approximately 70% lower odds of MS in males and 67% in females, consistent with its widely reported role in MS. These findings underscore the value of sex-stratified analysis in revealing exposure patterns that may inform future research on MS risk factors.

Thus, the strong negative associations of physical activity, vitamin D sufficiency, and EBV seronegativity highlight their potential relevance for clinical risk assessment. Lifestyle-related factors (physical activity, smoking, and alcohol) and biological markers (EBV and vitamin D) emerged as stronger independent indicators than psychological variables. An inverse gradient was observed for both physical activity and vitamin D, with higher levels corresponding to lower odds of MS. Clinical priorities include promoting physical activity, smoking cessation, and vitamin D repletion in MS risk mitigation strategies. The stronger psychological associations in females suggest that stress management and mental health screening are critical for female patients with MS. The sex-specific pattern observed for alcohol avoidance in males warrants further investigation to inform targeted preventive strategies. The significant vitamin D × stress interaction, although requiring validation, points to a potential synergistic pattern between these factors, particularly in females. This observation warrants further investigation in prospective studies to explore whether combined effects may contribute to MS susceptibility. These findings support a multifactorial approach to MS risk assessment, integrating lifestyle, biological, and psychological factors.[44,45] Detailed analyses of these risk factor categories will be explored in forthcoming publications to provide deeper insights into their specific contributions to MS etiology.

There were some limitations to this study. The case-control design precluded causal inference, and recall bias may have affected self-reported data (e.g., smoking, stress, and alcohol). Another limitation was the retrospective assessment of psychological variables (e.g., stress, anxiety, and depression) in patients with, while controls were assessed contemporaneously. This methodological discrepancy may have introduced recall bias, potentially exaggerating associations between psychological factors and MS. Although higher education remained significantly associated with MS in the multivariate model, it was not included in the interaction or sex-stratified models. This decision was made to maintain model parsimony and focus the analysis on modifiable lifestyle, biological, and psychological factors. Further studies may consider exploring the role of education more directly as a potential effect modifier or confounder. Nonconvergence in the interaction model suggests potential overfitting, limiting the reliability of interaction findings. Small subgroup sizes for some interactions (e.g., EBV-smoking) reduced statistical power. The study population may not fully represent diverse geographic or ethnic groups, limiting generalizability. The limited predictive capacity of the passive smoking model warrants caution in interpreting its association with MS risk.

In conclusion, longitudinal studies are needed to confirm causality, particularly for physical activity, vitamin D, and psychological factors. Mechanistic studies should explore the vitamin D × stress interaction to clarify its biological basis and resolve discrepancies with expected gradients. Larger, diverse cohorts are required to validate sex-specific findings and improve power for interaction analyses. This study underscores the multifactorial nature of MS, with physical activity, vitamin D, EBV status, smoking, alcohol, and psychological factors as key predictors. Sex-specific differences and interactions highlight the need for tailored prevention strategies. These findings lay the groundwork for future research and clinical approaches to reduce MS risk, with detailed category-specific analyses to follow in subsequent publications.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, analysis and/or interpretation, references and fundings: R.A., A.M., R.S.; Design, materials: R.A., R.S.; Control/supervision, critical review: R.S.; Data collection and/or processing: R.A., A.M.; Literature review: A.M., R.S.; Writing the article: R.A.

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