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Epstein-Barr Virus, Hygiene, and Multiple Sclerosis in Mexico City

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Abstract

Background/Aims: We studied whether serological antibody titers against Epstein-Barr virus (EBV) were related to multiple sclerosis (MS) risk in Mexico City, a population undergoing an epidemiological transition. Additionally, we explored the association between markers of hygiene and intestinal parasite infection and MS prevalence.

Methods: We conducted a hospital based case-control study in Mexico City; 52 incident MS cases. Childhood and adult hygiene scores were created using information from questionnaires. 51 cases and 51 controls provided blood samples for biomarker analyses.

Results: There was a 3-fold increase in risk of MS with each 1 unit increase in anti-EBNA1 titer (OR 3.02, 95% CI: 1.01-9.02). Increasing childhood and adult hygiene scores were associated with a modestly increased risk of MS ($OR_{childhood}$ 1.20, 95% CI: 1.04-1.38; OR_{adult} 1.28, 95% CI: 1.04-1.58). We found no association between antibody titer to neither Stronglyloides nor Ascaris and MS risk.

Conclusion: High anti-EBNA1 antibody titers appears to be associated with increased MS risk even in a region where MS had historically low incidence and infectious mononucleosis is rare, providing further support for the postulated role of EBV in MS etiology. We also found moderate support for a role of higher 'hygiene' being associated with susceptibility to MS.

Keywords: Epstein-barr virus; Multiple sclerosis

Introduction

There is compelling evidence for a role of the Epstein-Barr virus (EBV) in the etiology of multiple sclerosis (MS), [1,2] however, almost all the studies relating EBV infection to MS have been conducted in regions of high MS risk, such as the U.S., Canada, and northern Europe [2]. Whether similar findings apply to regions of low MS prevalence remains to be determined; a recent study in pediatric MS [3] did include a few children from Latin America and South Italy, but numbers were too small for any independent analysis. A study of EBV DNA in Mexico did not find an association between MS cases and controls [4], though in general results of studies on EBV viral load and risk of MS have been mixed [5].

We, therefore, chose to study whether serological antibody titers against EBV were related to MS risk in Mexico City, a large urban population undergoing a fast epidemiological transition. Data suggests that the incidence of MS has been rapidly increasingly in Mexico from the 1970's, when it was considered a rare disease, to the 1990's when it has been estimated to be the second most common reason for admission to a neurology ward [6]. Additionally, we also explored the association between markers of hygiene and intestinal parasite infection and MS prevalence in this developing country where parasitic infections are relatively common.

Methods

Study population

We conducted a hospital based case-control study in Mexico City in collaboration with the National Institute of Public Health of Mexico. Fifty-two incident MS cases were recruited from the outpatient MS clinic at the National Institute of Neurology and Neurosurgery (INNN) between February and May of 2010. Inclusion criteria for cases were 1) being at least 18 years old, 2) having a diagnosis of relapsing/remitting MS based upon the McDonald criteria within the last 2 to 5 years, with first symptoms occurring between ages 18 and 45 and 3) being a resident of Mexico City for at least the last year. Requiring residency for one year helped to establish a distinct study population and to reduce issues with referrals of individuals outside Mexico City to this hospital. One hundred twenty-five age- and sex-matched controls were selected in three ways: 1) non-MS patients presenting at the outpatient headache clinic of the INNN (n=43), 2) individuals accompanying MS patients or other patients of any clinic at INNN (n=74) (family members of patients of the MS clinic were excluded), 3) population-based controls chosen by matching the house addresses from a case and then randomly selecting a household on the same block (n=8). The number of population-based controls was small because this selection strategy was abandoned early in the study due to the large number of houses that had to be visited to recruit an eligible control. Inclusion criteria for controls included 1) having been a resident of Mexico City for at least the last year and 2) being over 18 years of age. Personal history and demographic information was collected by study interviewers who administered structured questionnaires. Serum was collected from all cases (n=51) and 1

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matched control per case (n=51) for biomarker analyses. Controls that did not provide serum were similar to those that did provide samples on personal and demographic information (example: age, sex, birth place, education, occupation).

Anti-EBNA1 Ab titers and total IgG

IgG antibody titer levels against the Epstein-Barr virus nuclear antigen 1 (anti-EBNA1) and total IgG were measured at the Children's Hospital Boston. IgG antibody titers were measured using a commercially available immunoturbidimetric assay on the Roche P Modular system (Roche Diagnostics; Indianapolis, IN); anti-EBNA1 titers were measured by ELISA (DiaMedix Corporation; Miami, FL) which was modified for quantitative analysis. The intra-assay CV was 1.47%.

Parasitic infections

IgG antibody titers against *Ascaris lumbricoides* and *Strongyloides stercoralis* were measured by ELISA which was developed in the laboratory of Dr. Irving G. Kagan at the Parasitic Disease Consultants [7]. IgG antibody titers to *Ascaris lumbricoides* had a reference range of: positive ≥ 1:32, reactive =1:16, and negative <0.09. *Strongyloides stercoralis* IgG antibodies had an optical density (wavelength 450 nm-650 nm) reference range of: positive ≥ 0.09, negative <0.09.

Concordance was 80% for the presence of antibodies against *Ascaris* and the inter-assay CV for a quantitative measure was 20.5%. Similarly, the concordance was 80% for the presence of antibodies against *Strongyloides* and the inter-assay CV was 16.1%. Additionally, data were collected via questionnaires regarding history (how many times in your life have you been infected with intestinal parasites?) and treatment (how many anti parasitic treatments have you ever taken?).

Hygiene score

A childhood hygiene score was created using information available from the questionnaire administered by interviewers. An individual's childhood hygiene score was the sum of the individual values for variables with higher scores corresponding to more hygienic environment. Components of the childhood hygiene score included: population size of birthplace (0=town or small city <100,000; 1=big city >100,000 inhabitants), crowding (number of people per bedroom: 0=9-20; 1=6-8; 2=1-5), number of siblings (0=8-14; 1=3-7; 2=0-2), ownership of home (0=rent; 1=own), floor of house (0=ground; 1=cement; 2=vinyl, wood, carpet), drinking water in house (0=no; 1=yes), electricity in house (0=no; 1=yes), bathroom (0=lake, ground level, black hole, latrine; 1=toilet with tray; 2=piped water toilet), self-reported history of parasitic infection(0=yes, 1=no), and highest level of education for study participant and parents (0=primary or secondary; 1=preparatory; 2=professional or post-graduate). Education levels were included to capture potential relevant exposures in childhood. 0 was the lowest childhood score an individual could receive and 19 was the highest. In the case of a missing variable, the missing value was assigned the most frequent response based on the distribution in the controls and indicators for missing values were included in the logistic regression models. There was little missing data for each component of the childhood hygiene score, the most missing data was only 9.6% for highest level of father's education.

As an exploratory analysis, an adult hygiene score was also created. It included variables associated with the individual's adult living situation. Components of the adult hygiene score were related to construction of the individual's current home: walls (0=cardboard, sheetrock: 1=brick, partition) and floors (0=ground, cement, vinyl tile; 1=ceramic tile, wood, carpet), as well as having the following items in their home (no=0, yes=1): piped water, radio, television, VCR, telephone, refrigerator, washing machine, oven, heater, computer, and car. The lowest adult hygiene score that could be calculated was 0 and the highest was 13.There was no missing information on any of the component variables in the adult hygiene score.

Multivariable logistic regression models were used to calculate odds ratios (ORs) of MS and 95% confidence intervals (CIs). To control for potential confounders, we included the following established MS risk factors: age in 5 year intervals, sex, and smoking status (ever vs. never).

Results

Study population characteristics

Characteristics of the study population are shown in Table 1. As expected, anti-EBNA-1 antibody titers were higher in MS cases than controls. The IgG count, titers of antibodies against *Ascaris* suggestive of current infection, and percent reporting a history of parasitic infection were all lower in individuals with MS compared to those without MS (Table 1).

The range of the childhood hygiene score in the controls was from 1 to 18 (median=9). The range of adult hygiene scores in the controls was from 3 to 13 (median=10). There was little correlation between childhood and adult scores (R^2=0.38). Because childhood hygiene was the primary exposure of interest we also looked at the characteristics of the control group by low vs. high childhood hygiene score (Table 2). There was a significant difference (p<0.05) between those in the low vs. high childhood hygiene score groups for age (younger among those

	Cases	Controls
N	52	125
Age (yrs)	34.5	33.7
Sex (% female)	63.5	64.8
Childhood hygiene score[a]	12.4	9.4
Adult hygiene score[b]	10.7	9.8
EBV (% seropositive)[c]	100	96.1
Anti-EBNA1 IgG Ab titerc	4.40	3.88
Infectious mononucelosis (% ever)	0	0.8
IgG (mg/dL)[c]	1668	1779
Cigarette smoking (% ever)	69.2	68.0
Parasitic infection (% ever)[d]	42.0	51.2
Number of parasitic infections(0-56)[d]	2.0	1.9
0 parasitic infections (%)	58.0	48.8
1-2 parasitic infections (%)	24.0	32.8
3-10 parasitic infection (%)	16.0	15.2
10+ parasitic infections (%)	2.0	3.2
Age of first intestinal parasitic infection(1-32)[d]	11.9	10.3
Strongyloides stercoralis antibodies (% positive)[c]	21.6	17.0
Ascaris lumbricoides antibodies (% positive)[c,e]	47.5	41.2
Past Ascaris (% positive)	37.3	29.4
Current Ascaris (% positive)	10.2	11.8

*mean values unless otherwise noted
[a]score based factors related to socioeconomic status during childhood and self-reported history of parasitic infection as described in Methods
[b]score based on factors related to current socioeconomic status as described in Methods
[c]serology based on 51 cases and 51 controls with serum samples
[d]self report
[e]total of past and current

Table 1: Characteristics of multiple sclerosis cases and controls.

Characteristics	Low hygiene score (<9)	High hygiene score (≥ 9)
Number	53	72
Age (years)[b]	35.8	32.1
Female (%)	67.9	62.5
Ever smoker (% yes)	62.3	72.2
Reported # of parasitic infections	2.3	1.6
Age at first parasitic infection (years)	10.4	10.3
Anti-parasitic treatment (% yes)	78.9	84.7
# anti-parasitic treatments[b]	6.4	10.3
IgG (mg/dL)[c]	1815	1745
Anti-EBNA1 Ab titers[c]	3.7	4.0
Ascaris (% positive)[c]	40.0	55.2
past *Ascaris* (% positive)	33.3	41.4
current *Ascaris* (% positive)	6.7	13.8
Strongyloides (% positive)[c]	6.7	27.6
Reported parasitic infection (% ever)	41.5	54.2
Education (%)[b]		
primary or secondary	83.0	20.8
preparatory	15.1	41.7
professional	1.9	37.5
Father's education (%)[b]		
primary or secondary	96.2	58.3
preparatory	1.9	16.7
professional	1.9	25.0
Mother's education (%)[b]		
primary or secondary	100	73.6
preparatory	0	8.3
professional	0	18.1
Owned home (% yes)	62.3	72.2
Siblings[b]	5.8	3.4
Crowding (#persons/bedroom)	5.4	2.7
Floor of house[b]		
ground	39.6	1.4
cement	52.8	56.9
tile, wood, carpet	7.6	41.7
Drinking water in home (% yes)[b]	50.9	98.6
Electricity in home[b]	73.6	100.0
Size of hometown, % big city (>100,000 inhabitants)[b]	13.2	76.4
Bathroom as a child, %[b]		
black hole, ground level, body of water	49.1	0.0
toilet with tray	37.7	15.3
piped water toilet	13.2	84.7

Mean values unless otherwise noted, totals may not sum to100% due to rounding
[a]Low and high hygiene scores based on median value.
[b]Significant difference between high and low hygiene score (p<=0.05)
[c]Based on 51 controls who provided serum samples. (low hygiene score: n=25; high hygiene score: n=26)
……..Variables below line included in childhood hygiene score

Table 2: Characteristics according to childhood hygiene scorea among controls.

with high hygiene score) and number of anti-parasitic treatments taken (higher among those with high hygiene score). Otherwise, no significant differences were seen for those with a high versus low childhood hygiene score (Table 2).

EBV and risk of MS

EBV seropositivity, assessed by a positive anti-EBNA Ab titer, was higher in cases compared to controls (100% vs. 96.1%). Only one individual (a control) in this study reported a history of infectious mononucleosis (compared to roughly 15% in the US) [8]. There was a greater than 3-fold increase in risk of MS with each 1 unit increase in anti-EBNA1 titer after adjustment for age, sex, EBNA1 titers, total IgG, smoking history, and childhood hygiene score (Table 3). We also adjusted for adult hygiene score and found little change in results (multivariable adjusted OR: 3.22, 95% CI 1.11-9.29). We further considered whether childhood and adult hygiene scores were associated with MS. Individuals with MS had a higher mean childhood hygiene score (12.4 ± 3.9) compared to controls (9.4 ± 4.2); as well as higher mean adult hygiene score (10.7 ± 2.2) compared to controls (9.8 ± 2.3). Increasing childhood and adult hygiene scores were associated with a modestly increased risk of MS; this association remained after adjustment for age, smoking, total IgG, and anti-EBNA1 titers (Table 3). We also performed analyses were including both childhood and adult scores in the model, and little change in the results were found. Additionally, we considered childhood hygiene score without study participant's or parents' education level and found similar increases in risk of MS as when education was included (multivariable adjusted OR: 1.27, 95% CI 1.06-1.52).

In exploratory analysis, we found no association between antibody titer to *Stronglyloides* and MS risk when considering the optical density of antibodies to *Strongyloides* either as positive vs negative (multivariable adjusted OR 1.26 95% CI: 0.34-4.64) or as a continuous variable (multivariable adjusted OR 0.38 95% CI: 0.03-5.51). The association was also null for risk of MS associated with antibodies to *Ascaris*. We compared *Ascaris* antibody response between those who were positive or reactive and those that were negative (multivariable OR 0.53 95% CI 0.21-1.37), those with a probable past *Ascaris* infection compared those with negative serology (multivariable OR 0.49 95% CI 0.18-1.31), and those with evidence of a current *Ascaris* infection and those who were seronegative (multivariable OR 0.79 95% CI: 0.16-3.95). Lastly, we investigated titers of antibodies to *Ascaris* as a continuous variable and risk of MS and found no association (multivariable OR 0.72 95% CI 0.35-1.45).

Discussion

In this case-control study based in Mexico City, we found a significant 3-fold increase in risk of MS with increasing serum anti-EBNA1 IgG antibody titer similar to reports from regions with high MS incidence [2]. We are limited in inferring temporality as anti-EBNA1

	OR 95% CI
childhood hygiene score[a]	
age adjusted OR	1.17 (1.06-1.29)
multivariable adjusted OR[d]	1.20 (1.04-1.38)
adult hygiene score[b]	
age adjusted OR	1.21 (1.02-1.44)
multivariable adjusted OR[d]	1.28 (1.04-1.58)
anti-EBNA-1 antibody titers[c]	
age adjusted OR	3.67 (1.33-10.13)
multivariable adjusted OR[b]	3.83 (1.36-10.78)
multivariable adjusted OR[e]	3.02 (1.01-9.02)

[a]OR for 1 point increase in childhood hygiene score. A high hygiene score corresponds to no parasitic infections and a higher socio economic status in childhood
[b]OR for 1 point increase in adult hygiene score. A high hygiene score corresponds to a higher socio economic status in adulthood
[c]OR for 1 unit increase in anti-EBNA1 titer. Includes 51 cases and 51 controls had serum samples
[d]Adjusted for age, sex, EBNA1 titers, total IgG, and smoking history
[e]Adjusted for covariates in d and childhood hygiene score

Table 3: Odds ratios of multiple sclerosis by hygiene scores and anti-EBNA1 antibody titers.

Ab titers were measured after MS diagnosis. However, the magnitude of the effect of increasing anti-EBV antibody titers is similar to other prospective studies with anti-EBNA1 Ab titers measured well before disease onset [2], and, anti-EBNA1 Ab titers tend to remain stable over one's lifetime [9] suggesting these levels likely reflect Ab titer before disease onset. These findings are consistent with a postulated role for EBV infection in MS etiology. Despite the notable differences in population characteristics and pathogen exposures between Mexico and North America and Europe, the finding of a similarly strong association between anti-EBNA titer and MS provides further support the epidemiologic data. The rarity of mononucleosis in this population is consistent with the younger age at EBV infection generally noted in developing or middle income countries as compared with the U.S. and Europe [10]. Though this could also be attributable to lack of reporting or diagnoses.

We also found that a childhood hygiene score which included self-reported history of parasitic infection and markers of socio-economic status in childhood was associated with a modest increase in MS risk. In exploratory analyses, we also found our adult hygiene score which accounted for factors related to socioeconomic status in later life to be modestly associated with MS risk. Although these hygiene scores were associated with MS risk, neither the presence of antibody titers against *Ascaris* or *Stronglyodies* nor self-report of past parasitic infection or treatment had significant links to risk of MS. Some prior data suggests a favorable impact of parasitic infection on disease course among MS patients [11-13], and helminth infection has been shown to bias the immune system towards Th2 and regulatory T-cell responses [14]. However, due to our small sample size and the potential misclassification of exposure for both the self-reported and serological measures, it is difficult to draw conclusions regarding parasitic infection. The role of 'hygiene' in explaining the increasing incidence of MS worldwide [15] is still unclear but is supported by the apparent increase in MS risk in regions and countries undergoing the so called "epidemiological transition", which is characterized by improved sanitation with reduction of intestinal parasites and other infectious diseases, and an increase in incidence of chronic diseases [11,16]. Examples include an apparent increase in MS incidence in Sardinia and other southern European regions [16]. There are, however, several correlates of 'high hygiene' and it is possible that another factor may explain an observed association between hygiene and MS. In addition, it is important to note that although young age at EBV infection is commonly reported in developing countries [10], the markers of "low hygiene" used in our childhood and adult hygiene scores are likely capturing exposures that could be independent factors in the etiology of MS.

Limitations of this study include the use of hospital instead of population based controls; however because the selection of controls was unlikely jointly associated with exposure of EBV and outcome of multiple sclerosis the chance for selection bias is small. We also controlled for a number of potential confounders of the association between EBV and risk of MS. In addition, as in all studies, the data obtained from questionnaires is susceptible to misclassification; unless associated with both exposure and outcome, however, such misclassification would most likely result in a bias towards the null, and it is thus unlikely to explain the results of the study.

The primary finding of this investigation is that high anti-EBNA1 Ab titers appears to be associated with increased MS risk even in a region where MS had historically low incidence and infectious mononucleosis is rare, providing further evidence of a role for EBV in MS etiology. We also found moderate support for a potential role of higher 'hygiene' being associated with susceptibility to MS.

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