Journal of the Neurological Sciences 325 (2013) 86–89

Contents lists available at SciVerse ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Association of Epstein–Barr virus infection with multiple sclerosis in India

Lekha Pandit [a,*], Chaitra Malli [a], Anita D'Cunha [a], Rajesh Shetty [b], Bhim Singhal [c]

[a] Center for Advanced Neurological Research, Nitte University, Mangalore, Karnataka, India
[b] Dept of Neurology, AJ Hospital and Research center, Mangalore, Karnataka, India
[c] Department of Neurology, Bombay Hospital and Research Center, Mumbai, India

ARTICLE INFO

Article history:
Received 3 November 2012
Received in revised form 30 November 2012
Accepted 5 December 2012
Available online 9 January 2013

Keywords:
Epstein–Barr virus
Multiple sclerosis
Indian population

ABSTRACT

Objectives: Epstein–Barr virus (EBV) seroprevalence is high from early childhood in Indian populations, though multiple sclerosis (MS) is uncommon. The present study aims to evaluate the association of EBV infection with MS in Indian patients.
Method: In this study 140 MS patients and equal number of matched controls were included. Estimation of serum Immunoglobin G (IgG) for EBV Nuclear antigen 1 (EBNA1), viral capsid antigen (EBV-VCA) and early antigen (EB-EA) were obtained by quantitative enzyme linked immunosorbent assay (ELISA). Patients and controls were genotyped for the human leukocyte antigen (HLA) *DRB1*1501* allele.
Results: A modest difference was observed for EBNA1 ($p=0.02$) and EBV-VCA ($p=0.03$) titres in MS patients as compared to healthy controls. There was no association between EBNA1 titres and MS. High EBNA1 titre (>99.75 U/l) was significantly associated with *HLA DRBI*15:01* (OR = 4.92. CI = 1.07–22.57) status in MS patients but not in healthy controls (OR = 1.19, CI = 0.53–2.63).
Conclusion: Evidence for a strong association with remote EBV infection was lacking in this study of Indian patients with MS. Patients who are carriers of *HLA DR15* allele may have high EBNA1 titres. These preliminary results need to be reproduced in an independent and larger dataset.

1. Introduction

Multiple sclerosis is a chronic immune mediated disorder of complex etiology. Both environmental and genetic factors are implicated in disease causation. Studies in patients of Northern American descent show that nearly 99% of adults with MS have been infected with EBV as compared to 94% age matched controls [1]. In pediatric cases, the association between EBV infection and the development of MS is not very clear. In a North American study, 108 (86%) children with MS were seropositive for remote EBV infection, compared with 61 (64%) matched controls [2]. Pohl et al. in their study found a much stronger association with 98.6% of pediatric MS and 72.1% healthy children showing evidence of remote infection with EBV [3]. These data suggest that prior EBV infection may be a prerequisite for the development of disease and that timing of infection may be important. It is not clear whether specific risk factors important for MS in regions of high prevalence are relevant in areas where the disease is less prevalent. The declining risk in migrants from regions of high to low MS prevalence suggests regional differences in the nature of environmental risk factors [4,5]. Some studies propose that EBV strain variations may alter susceptibility to MS [6,7] and that geographic differences in MS prevalence may be linked to EBV as a result.

In India the role of environmental agents involved in MS has not been studied in detail. A study of 63 MS cases and matched controls from the Mumbai region has shown a significant association with childhood viral infections and co-existence of other autoimmune disorders [8]. However the specific role of EBV has not been studied in association with MS in India. In the present investigation we sought to determine whether EBV plays a role in MS risk in the Indian population.

2. Materials and methods

2.1. Patients

In this study 136 relapsing (RR) and 4 secondary progressive (SP) MS patients were included. Diagnosis of MS was made by McDonald [9] criteria. Among the 140 MS cases, 48 (34%) were from Mumbai (author B.S.S.) and the remaining 92 (66%) were patients from the Mangalore demyelination registry. This registry was established in 2007 and maintained by L.P. & R.S. The purpose was to establish a database of all central nervous system demyelinating disorders seen by neurologists in the coastal city of Mangalore, in southern India. Patients entered into this registry are re-evaluated and remain on long term follow up. Mumbai and Mangalore are cities on the western coast of India 900 km apart. Demographic details, year of onset of disease, type of MS or non-MS disorder, disease status in MS (relapse or remission) and time of blood sample collection after disease onset

* Corresponding author at: KS Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India. Tel.: +91 824 2204471.
E-mail address: panditmng@gmail.com (L. Pandit).

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http://dx.doi.org/10.1016/j.jns.2012.12.004

were noted. Past history of infectious mononucleosis (IM) was enquired about.

2.2. Controls

Healthy controls included in this study were age (±1 year) and sex matched controls. These included volunteers or those who visited the neurology clinic for non-demyelinating neurological disorders such as headache, low back pain and carpal tunnel syndrome.

2.2.1. Serum antibody detection and quantification

Blood samples were processed within 2 h of collection, sera separated, aliquoted and stored at −20 °C. Samples from Mumbai were couriered by air in bulk to reach the analysis lab of first author at Mangalore within 6 h of dispatch where they were stored at −20 °C till time of assay. Serum Immunoglobin G (IgG) for EBV Nuclear antigen 1 (EBNA1), viral capsid antigen (EBV-VCA) and early antigen (EBV-EA) were measured by quantitative ELISA (Virion Serion, Wurzberg, Germany) as per manufacturer's instructions. Antibody titres against cytomegalovirus (CMV) were also determined to assess the specificity of any association that may be found between MS and EBV serology. Optical density values were measured using a Chemwell 2910 automated EIA analyzer (Awareness Technology, USA) for calculation of units per milliliter (U/ml). Results were deemed positive if values were >3 U/ml for EBNA1, >15 U/ml for EBV-EA & VCA and >40 U/ml for CMV IgG, respectively. This assay reliably measures EBNA and EBV-VCA IgG levels up to 200 U/ml. All high titres were truncated at this level. Patient and matched control samples were analyzed blinded to case status. The intra-assay coefficients of variation were VCA — 2.9%; EBNA1 — 2.9%; EA — 3.8% and CMV — 7% antibodies, respectively. This study was approved by the institutional ethical committees of participating centers at Mangalore and Mumbai.

2.2.2. HLA DR typing

HLA DR typing was performed by polymerase chain reaction (PCR) with sequence specific probes [10]. Alleles that were *DRB1*1501/1502* positive by this low resolution typing technique were sequenced [11] for accurately determining *HLA DRB1*1501* status.

2.3. Statistical analysis

Analysis was performed using SPSS version 20.0 (IBM Corporation, Armonk, NY). For univariate analysis, Wilcoxon rank sum test was used for comparing antibody titres for EBNA1, EBV-VCA and EBV-EA between MS patients and matched controls. For stratification by anti-EBNA1 antibody titres within patients and controls, antibody titres were divided into quartiles of the distribution among controls (Quartile 1 = <22.5, Quartile 2 = ≥22.5–<50.5, Quartile 3 = ≥50.5–<99.5, Quartile4 = ≥99.5 U/l) and recoded. *HLA-DR15* was considered as a dichotomous variable (positive/negative). In keeping with the nested case–control model of the study, we used Cox regression analysis (dependent variables were coded with cases having higher value than controls and additionally a status variable was included) for evaluating the risk association between antibody titres, *HLA-DR15* and MS. Antibody titres for EBNA1 were further stratified for *HLADRB1*15:01* status in MS patients and controls. Logistic regression was performed to determine whether risk of MS associated with EBV infection was modified by genotype. Odds ratio (OR) and 95% confidence interval (CI) were calculated.

Table 1
Clinical demographics.

	MS	Controls
Gender F (%)	82 (59%)	82 (59%)
Age (mean ± SD)	36.1 ± 11	36.7 ± 10
Disease course	RR = 136 SP = 4	NA
Disease duration at time of sampling (median years)	5.4 ± 3.2	NA

RR = relapsing remitting and SP = secondary progressive.

Table 2
Comparison of EBV antibody titres between MS patients and controls.

EBV IgG titre (Median & IQR)	MS patients	Matched controls	*p* value
EBNA1 IgG	63.75 (21.5–137.5)	42.00 (22.1–89.3)	0.02
EBV-VCA IgG	200 (110–200)	158 (71.2–200)	0.03
EBV-EA IgG	4.40 (2.2–9.0)	3.70 (2.0–8.5)	0.19

3. Results

3.1. Demographics

This study included a total of 140 MS patients including 136 cases of relapsing remitting (RR) and 4 cases of secondary progressive (SP) MS (Table 1). An equal number of healthy individuals (relatives or patients with minor neurological illnesses) matched for age and sex were included as controls. Only 2 MS patients gave a history of infectious mononucleosis and none in healthy controls.

3.2. Viral antibody prevalence

There were no significant differences in antibody prevalence between MS and matched control samples tested. Anti-EBNA1 IgG was seen in 98.5% MS, and 96.4% healthy controls. Antibody prevalence for EBV-VCA was 97.9% in MS samples, as compared to 98.6% healthy controls. EBV-EA IgG was detected in 12.8% MS as compared to 11.8% controls. Among MS patients who were EBV-EA IgG positive only 8/18 patients had a clinical relapse close to the period of blood collection.

3.2.1. EBV viral antibody titres and association with MS

Antibody titre for EBNA1 (50th median and interquartile range) showed a statistically significant though modest difference (Table 2) between 140 MS patients and matched controls ($p = 0.02$). A similar result was observed on comparing patients and controls for EBV-VCA ($p = 0.03$) antibody titres. Anti-EA IgG titres showed no variation between the groups. Mumbai and Mangalore samples were analyzed separately or both MS patients and controls and found to have no significant differences (data not shown). Anti-CMV IgG titres were similar

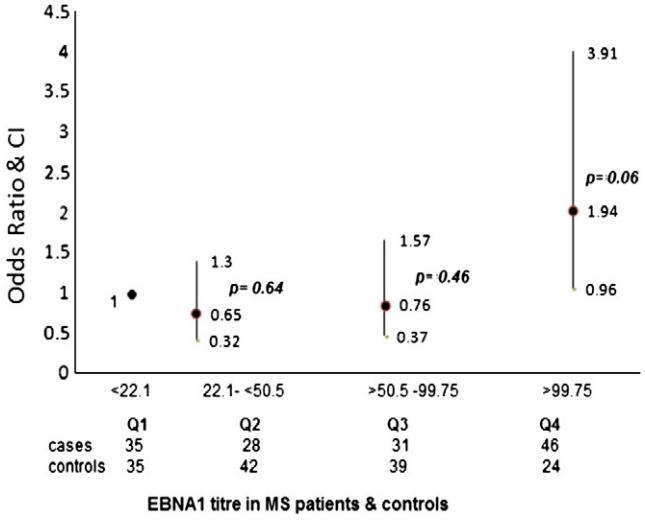

Fig. 1. Multiple sclerosis risk association with EBNA1 titre.