Original Article

Evaluating the Role of HLA DRB1 Alleles and Oligoclonal Bands in Influencing Clinical Course of Multiple Sclerosis – A Study from the Mangalore Demyelinating Disease Registry

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Abstract

Background: The possible interaction between genetic and immunological factors in influencing clinical course of multiple sclerosis (MS) has not been studied previously in Indian population. **Aim:** In this study we evaluated the association of HLA alleles and OCB in affecting clinical course and disability of MS. **Methods:** Clinical and demographic features of 145 MS patients who had CSF oligoclonal bands (OCB) tested by isoelectric focussing technique were analyzed, disability status estimated, and HLA DRB1 alleles were genotyped. **Results:** OCBs were positive in 53.8% (78/145) of all MS cases. Patients with CSF OCB had more frequent relapses and an association with HLA DRB1*15. Early disease onset and a high annualized relapse rate was associated with HLA DRB1*03 allele. A relapsing remitting course for MS was seen with HLA DRB1*03 & 15 while a progressive disease was associated with DRB1*01. Presence of both OCB and HLA DRB1*13 was significantly associated with disability in this cohort. **Conclusion:** The results of our study suggest that an interaction between immunological and genetic factors may influence disease onset, course, and disability in MS.

Keywords: HLA DRB1 alleles, multiple sclerosis, oligo clonal bands

Introduction

Multiple sclerosis (MS) is a chronic autoimmune inflammatory demyelinating disorders of the central nervous system (CNS) occurring worldwide. Prevalence of MS in India is low compared to white populations of Europe and North America. A population-based prevalence study from southern India has shown a prevalence of 8.3/100,000 for MS.[1] The etiopathology of the disease is unclear and possibly involves complex interactions between environmental and genetic factors.

Immunological studies have shown that genes within the human leukocyte antigen (HLA) region account for the strongest genetic risk for MS. Among Indians the genetic susceptibility for MS appears similar to white Europeans. The strongest association signal comes from HLA-DRB1*1501 in the class II region.[2-4] Analysis of genetic variations outside the major histocompatibility complex has also shown an overlap between Indian and European populations.[5,6] Our published studies have shown that environmental factors such as smoking and remote Epstein–Barr virus (EBV) infection which are identified risk factors in Caucasian populations are not associated with disease among Indian populations.[7]

The HLA complex is not only associated with disease susceptibility, but also may influence clinical and immunological features in patients with MS.[8] The mechanism by which such influences might occur is believed to be the interdependence between the HLA-DRB1 genotype and the phenotypic status of the oligoclonal immunoglobulin G (IgG) bands (OCBs).[9] Oligoclonal bands (OCBs) of immunoglobulin G (IgG) are detected in the CSF of significant number of MS patients and supports the diagnosis.[10,11] Oligoclonal band (OCB) patterns differ between patients but remain constant during disease course[12,13] and are believed to reflect the intrathecal synthesis of IgG antibodies.[14,15] A study conducted by Rand *et al*. suggests that in a subset of MS patients, Epstein–Barr virus nuclear antigen 1 (EBNA-1) may be a major target of selected OCBs.[16] Interestingly our study and those of others have shown that patients who are carriers of HLA DRB1*1501 allele may have high EBNA1 titres, underscoring the intimate relationship of environmental and genetic influences in MS pathogenesis.[17,18]

In this study we aimed to characterize the clinical course of MS patients in relation to the presence of OCBs and HLA DRB1 allele status in a subset of patients belonging to a dedicated demyelinating disease registry in south India.

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Materials and Methods

Patient selection

In this study 145 consecutive MS patients who had lumbar puncture performed as part of their investigations were enrolled from our registry.[19] The diagnosis of MS was made according to the revised McDonald 2017 criteria.[11] All had been tested for the presence of OCBs in paired samples by isoelectric focusing (IEF) electrophoresis prior to started disease modifying therapy.[10] This study was approved by the Institutional ethics committee and informed consent was obtained before study entry.

Evaluation of clinical course

Clinical characteristics including type of MS, gender, age at disease onset, disease duration, and treatment status were noted [Table 1]. The annualized relapse rate (ARR - calculated as number of attacks/duration of disease) and the most recent expanded disability status score (EDSS) were calculated for all patients.

Oligoclonal band testing

Paired serum and CSF samples were analyzed using Hydrasys 2 Sebia (France) system as per manufacturer's instructions. Interpretation of the results was made according to the 5 classic banding patterns described.[10] Pattern 2 (presence of bands only in CSF) and 3 (presence of bands in CSF and serum with additional bands in CSF) were interpreted as positive results and indicated intrathecal IgG synthesis.

HLA-DR typing

HLA-DRB1 typing was performed by polymerase chain reaction (PCR) with sequence-specific probes.[4,20]

EBNA1 IgG affinity immunoblotting test

It was performed in a subset of 21 patients (RRMS- 16, SPMS -5). Serum and corresponding CSF IgG of each patient was initially purified (Nab Spin Kit, Thermo Fisher Scientific). Immunoblotting was done using previously published protocol.[16]

Statistical analysis

Data analysis was performed using the IBM SPSS for Windows Version 21.0 (IBM Corp.;Armonk, NY, USA) statistical package. We compared mean age at onset of MS in different groups using the Student *t*-test. The comparisons of other variables (disease duration, EDSS score and annual relapse rate) were based on the Mann–Whitney test. The χ^2 test was used to compare the qualitative variables and to estimate possible correlations. Odds ratios (OR) with 95% confidence interval (CI) were calculated when searching for the associations. The significance level of *P* values < 0.05 were considered as statistically significant. The relationship between measure of disability (EDSS) and other variables including immunological, genetic, and clinical parameters were assessed by logistic regression modelling.

Results

Among 145 MS patients, 77.9% (113/145) were relapsing remitting MS (RRMS), 16.6% (24/145) were secondary progressive MS (SPMS), and 5.5% (8/145) were primary progressive MS (PPMS). The demographic features are listed in Table 1. Ninety one patients (62.7%) were female (gender ratio F: M - 1.7:1). Mean age at disease onset was 28.5 ± 9.5 years and mean EDSS score was 3.06 ± 2.14 [Table 1]. All patients took treatment with disease modifying agent or immune suppressive therapy for median period of 8.0 years (5.0–11.0) from the time of diagnosis.

OCBs were positive (+) in 53.8% (78/145) of all MS cases. The predominant OCB pattern detected was pattern 2 and was seen in 85.90% (67/78). Patients were stratified based on positive OCBs [Table 2]. Noticeably disease duration was significantly longer (*P* - 0.003) in the OCB negative (-) patients (10.4 ± 5.7) when compared to OCB positive (7.8 ± 4.4). Annualized relapse rate was significantly more frequent (*P* - 0.02) in OCB-positive MS patients (0.48 ± 0.33) as compared to OCB-negative (0.39 ± 0.31) patients. Mean EDSS scores were similar between the two groups. Patients who were OCB positive (14%) did not have higher disability scores (EDSS ≥ 6) than those who were OCB negative (13%, *P*- 0.91).

We then looked at demographics and clinical course among MS patients in relation to their HLA DRB1 alleles. Age at onset was lower in DRB1*03 positive patients (23.7 ± 5.89)

Table 1: Demographic and clinical data of MS (*n*=145) patients

Factors	Value
Female (91): male (54)	1.7:1
Mean age	32.06±10.16
Disease course	
Relapsing-remitting MS	113 (77.9%)
Primary progressive MS	8 (5.5%)
Secondary progressive MS	24 (16.6%)
Age at disease onset	28.46±9.52
Disease Duration	9.05±5.22
Annualised Relapse Rate (ARR)	0.45±0.34
EDSS score	3.06±2.14

Table 2: Clinical features in MS patients based on presence or absence of Oligoclonal bands

Factor	OCB-positive *n*=78	OCB-negative *n*=67	*P*
Age at onset Mean±SD	27.48±9.16	29.59±9.88	0.196
Gender (F)	50 (64.1%)	41 (61.2%)	0.73
Disease duration Mean±SD	7.8±4.4	10.4±5.7	**0.003**
Annualised relapse rate Mean±SD	0.48±0.33	0.39±0.31	**0.02**
Current EDSS score Mean±SD	3.1±2.3	3.05±1.95	0.55
RRMS *n* (%)	60 (77%)	53 (79%)	0.84
SPMS *n* (%)	10 (12.8%)	14 (21%)	
PPMS *n* (%)	8 (10.2%)	-	

when compared to DRB1*03 negative patients (30.01 ± 10.10; *P* - 0.05). Frequency of HLA DRB1 alleles among MS subsets was analyzed [Table 3]. HLA DRB1*01 was most frequently associated with SPMS than RRMS (17% vs 1%; *P* - 0.002). The most frequent alleles associated with RRMS were HLA DRB1* 03 (11% vs. 0%; *P* - 0.03) and HLA DRB1*15 (47.7% vs 21.7%; *P* - 0.02). This association persisted when data was stratified for presence of OCB (data not shown). HLA DRB1*15 frequency was significant in OCB positive 42 (54%) versus OCB negative is 24 (36%) *P* = 0.04, OR = 0.47 (0.24–0.93).

A two-way analysis of variance (ANOVA) test was done to evaluate the effect of OCB status and HLA allele frequency on disability scores [Table 4]. Disability was significant in OCB positive patients who had HLA DRB1*13 allele when compared to OCB negative MS, (EDSS 10.0 ± 0.0 vs. 3.77 ± 1.12; *P* - 0.001). There were no significant associations between other alleles, presence of OCB, and disability.

We also estimated the impact of immunological, genetic, and clinical variables on disease progression [Table 5] using logistic regression models. Age at onset of disease and disease duration were independent and significant factors for progression of disability with each newly added HLA DRBI allele. In addition HLA DRB1*13 showed a significant association with disability progression independent of all other factors.

Among the 21 patients in whom EBNA1 IgG affinity immunoblot was done, there was evidence of intrathecal IgG synthesis for EBV in 28.5% (6/21). There was no difference in disability scores in positive patients (EDSS - 2.58 ± 2.93) when compared to Immunoblot negative patients (3.67 ± 3.69; *P* - 0.60). A third of these patients (2/6) were HLA DRB1*1501 positive.

Discussion

Among Indian patients with MS, HLA DRB1*15 followed by DRB1*03 have been significantly associated with disease susceptibility. In the 80s, published reports on MS from India have shown low CSF OCBs (30%).[21] More recently in a small study that included OCB as a diagnostic criterion, OCB was detected in 16/18 (85%) patients with MS.[22] Refinement of diagnostic criteria (which removed "optical spinal variants") and better techniques for OCB testing have probably

Table 3: Frequency of HLA DRB1 alleles in relapsing remitting (RR) MS and secondary progressive (SP) MS patients

HLA DRB1* analysis (*n*=61)	RRMS (*n*=44) *n* (%)	SPMS (*n*=17) *n* (%)	OR (95%CI)	*P*
01	1 (1%)	6 (17%)	17.7 (2.0-154.0)	0.002
03	10 (11%)	0	0.68 (0.61-0.78)	0.03
04	12 (14%)	4 (11%)	0.81 (0.24-2.6)	0.73
07	9 (10%)	3 (9%)	0.81 (0.21-3.19)	0.76
08	2 (2%)	0	0.71 (0.63-0.79)	0.36
10	2 (2%)	0	0.71 (0.63-0.79)	0.36
11	4 (5%)	1 (3%)	0.61 (0.06-3.6)	0.66
12	4 (5%)	1 (3%)	0.61 (0.06-3.6)	0.66
13	6 (7%)	4 (11%)	1.7 (0.46-6.59)	0.4
14	7 (8%)	6 (17%)	2.3 (0.73-7.6)	0.14
15	21 (47.7%)	17 (21.7%)	0.39 (0.17-0.87)	**0.02**
16	2 (2%)	0	0.71 (0.63-0.79)	0.36

Table 4: HLA DRB1 allele, oligo clonal bands and their interaction to disability (two-factorial analysis)

HLA DRB1 alleles value to disability			OCB value to disability		HLA DRB1 alleles and OCB interaction value to disability
HLA DRB1*	EDSS score	*p*	EDSS score	*p*	*p*
01	5.5±2.43 VS 3.88±1.88	0.04	6.0±0.7 VS 5.3±3.09	0.52	0.93
03	3.25±0.75 VS 4.02±1.9	0.35	3.5±0.0 VS 3.2±0.84	0.62	0.94
04	3.9±1.6 VS 3.97±1.98	0.37	2.8±0.97 VS 4.3±1.6	0.46	**0.03**
07	3.95±2.2 VS 3.96±1.91	0.9	4.8±3.3 VS 3.4±0.85	0.13	0.39
08	2.0±0 VS 3.9±1.91	0.18	0.0 VS 2.00		
10	3.0±0.7 VS 3.96±1.91	0.45	2.5±0.0 VS 3.5±0.0	0.86	0.58
11	4.5±1.2 VS 3.9±1.96	0.53	4.1±1.2 VS 5.0±1.4	0.85	0.46
12	3.4±4.3 VS 3.98±1.92	0.37	2.5±0.0 VS 3.6±2.5	0.77	0.46
13	4.4±2.2 VS 3.92±1.91	**0.004**	**10.0±0.0 VS 3.77±1.12**	**0.001**	**0.004**
14	4.7±2.01 VS 3.87±1.91	0.056	0 VS 4.7±2.01	-	-
15	3.7±2.05 VS 4.1±1.9	0.23	4.4±2.1 VS 2.9±1.8	**0.04**	0.078
16	3.75±0.35 VS 3.9±1.95	0.84	4.0±0.0 VS 3.5±0.0	0.72	0.99

Table 5: Logistic regression model for disability prediction in MS (comparison of EDSS scores against OCB presence, HLA DRB1 alleles, age, gender, disease duration and relapse rate)

Factor	B (regression coefficient)	OR (95%CI)	*P*
OCB	-1.5	0.21 (0.05-0.99)	**0.05**
HLADRB1*13	2.17	8.7 (1.65-46.7)	**0.01**
Gender	-0.16	0.85 (0.23-3.1)	0.80
Age at onset	-0.17	0.84 (0.77-0.92)	**<0.001**
Disease duration	-0.28	0.75 (0.66-0.86)	**<0.001**
ARR	-0.67	0.51 (0.09-2.7)	0.44

contributed to improved detection. MS was diagnosed in our registry using McDonald 2017 criteria,[11] and from among them 145 patients who had consented to a lumbar puncture were included and 53.8% had CSF OCBs.

The impact of OCB on clinical course of MS is not clear. While some studies show that presence of OCB is associated with a more aggressive disease course and disability progression[23-25] others suggest a more benign course.[26] Our study suggests that OCB-positive patients have higher annualized relapse rate. Patients who were OCB negative had a longer disease duration compared to those without and this suggests the possibility that OCB may appear early in the course of MS. However, OCB-negative MS patients resembled OCB positive patients in several aspects and included female preponderance, similar age at onset and disease course.

While several studies have established the role of HLA DRB1 alleles in influencing the risk of acquiring MS,[3,4] it is unclear whether there is a role in altering disease course and disability. HLA DRB1*15 has been reported in some studies to be associated with earlier onset of disease and poor outcome.[3,27,28] HLA DR B1*04 has also been linked to MS disability.[29] In our study immunogenetic associations were detected which suggested an influence on clinical features of MS. Early age of disease onset and a relapsing remitting course was associated with DRB1*03, while an increase in annualized relapse rate was noted in carriers of both HLA DRB1*03 and DRB1*15. Progressive MS was seen more often in patients carrying HLA DRB1*01. OCB-positive MS was most often associated with HLA DRB1*15. Disability was significantly associated with patients who were OCB positive and carried HLA DRB1*13 allele. HLA DRB1* 13 has been identified in previous studies as an important susceptibility allele,[30] but its role in disability was not studied. Age at onset and disability status were significant factors associated with disability, independent of HLA alleles and OCB presence and has been previously described.[31]

Antigen-specific banding pattern for EBNA-1 was observed in 6/21 (28.5%) MS patients, indicating there is an evidence of intrathecal IgG synthesis for EBV in small number of patients as previously described.[19] Some studies[16] suggest that in a subset of MS patients, EBNA-1 may be a major target of selected OCBs. There is however no evidence that its presence indicates a role for an EBV-driven chronic infection in MS. It is more likely that EBV-specific intrathecal oligoclonal IgG production seen in a subset of MS patients may be a part of humoral response driven by chronic brain inflammation.

In conclusion, in our study cohort, 53.8% of MS patients tested prior to initiating treatment, had CSF OCBs. These patients resembled OCB-negative patients in all respects except that had more frequent relapses. There were distinct genetic associations noted with disease onset, high relapse rate, disability progression, and presence of OCB. The results of our study suggest that presence of OCB and the HLA DRB1 allele subtypes may influence clinical course of MS. Our study was limited by the small number of patients included. A future study including a larger patient cohort is needed to determine the effect of immunogenetics and OCB in determining clinical course and disability in MS among patients in India.

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Conflicts of interest

There are no conflicts of interest.

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