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The significance of oligoclonal bands in multiple sclerosis: Relevance of demographic and clinical features, and immunogenetic backgrounds

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ABSTRACT

There has been no data on oligoclonal IgG bands (OCBs) for Turkish MS population, who is believed to be placed between Western and Eastern world regarding the clinical and immunological features. In the present study, we examined the correlation between the frequency of OCB and clinical and demographical features of MS in Turkish MS population. Our objective was to determine whether, population with OCB-positive and OCB-negative MS constitutes distinct subpopulations in terms of clinical, demographic and genetic base. A total of 210 clinically definite MS patients were included in the study. Patients were assessed clinically at baseline, 1 month later, every 3 months, and at year 5. No CSF OCB could be detected in 30 (14.3%) cases. 141 of the 156 female patients (90.4%) and 39 of the 54 male patients (72.2%) were positive for OCB. The female to male ratio was higher in the OCB-positive than in the OCB-negative group ($p=0.007$). Clinical course and disability were similar in the two patient groups. But mean relapse severity was higher in patients without OCBs in CSF. EDSS values got worse in OCB-negative group in year 5 ($p=0.008$). EDSS was also significantly higher in OCB-negative group in year 5 ($p=0.003$). There was no statistically significant difference regarding the usage of disease modifying therapy between the two groups. HLA DR15 antigen frequency was statistically higher in the OCB-positive than in the OCB-negative patients ($p=0.007$) and control group ($p=0.0002$). In conclusion, our results suggested that, MS patients with CSF OCBs have a female predominance, better clinical course with less disability and better prognosis, are associated with HLA-DR15.
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1. Introduction

Oligoclonal IgG bands (OCBs) have been characteristically present in the cerebrospinal fluid (CSF) of most of the clinically definite multiple sclerosis (MS) patients in Western populations (Andersson et al., 2006; Tourtellotte, 1988; Miller et al., 1983), and are believed to reflect the intrathecal synthesis of IgG antibodies. However, in Eastern countries the positivity of OCB has been reported to be much lower; which has resulted in diagnostic problems (Nakashima et al., 2002). MS is less common in Eastern population than in Western countries, and the clinical features of MS in this population have been distinguished by a high incidence of optic neuritis and acute transverse myelopathy (ATM) (Fukazawa et al., 1993b). Furthermore, increased IgG may be of less diagnostic value for MS in Eastern population, i.e. Japanese, than in Western countries (Fukazawa et al., 1993a,b), and the OCB-negative rate appears to be higher in Japanese MS patients (Kira et al., 1996). Despite the apparently high frequency of OCB-negative MS in Japan, data are lacking as to whether OCB-negative MS is merely a subtype of MS – which is relatively common in Japan – as another disorder distinct from MS, or merely reflects differences in immune response in the same disease between different races. On the other hand, in MS patients, OCBs can appear months after the first negative result (Zeman et al., 1996a,b).

It is unknown whether CSF antibodies have harmful or protective effects in MS. Based on a previous study (Zeman et al., 1996a,b), the prognosis of OCB-negative patients with MS is believed to be more favorable than that of OCB-positive patients.

There has been no data regarding the clinical and immunological features for Turkish MS population which is believed to be placed between Western and Eastern world. Thus, in the present study, we examined the correlation between the frequency of OCB and some selected clinical and demographical features of MS in Turkish MS population. As it has not been studied previously we aimed to determine whether, population with OCB-positive and OCB-negative MS constitutes distinct subpopulations in terms of clinical, demographic and genetic base, which has never been studied.

2. Materials and methods

A total of 210 MS patients were included in the study. All MS patients fulfilled the diagnostic criteria for definite MS according to Poser et al. (1983). They also fulfilled the criteria of McDonald et al. (2001). Patients were assessed clinically at baseline, 1 month later, every 3 months, and each time the patient suspected a new relapse.

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The clinical assessment was also performed at year 5. The usage of disease modifying therapy (DMT) at year 5 was also compared in each group.

Demographic and clinical data were shown in Table 1. The CSF samples were obtained by lumbar puncture with simultaneous serum sampling, and were stored at −80 °C. Patients underwent repeated CSF examination, not less than 1 year, when they were OCB-negative at the first examination. Mean age and mean duration of disease at first CSF examination were 34.9 years and 3.8 years, respectively. Each patient was assessed with Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983) by well-trained neurologists at scheduled follow-up visits. Relapse severity was assessed in two ways: the worst EDSS score at any relapse period, and recovery following relapse, which was assessed by EDSS difference between the documented attack period and one month after the attack. The physician assessing EDSS score of patients was blinded to OCB results and vice versa.

Patients on steroid treatment or other immunosuppressive drugs within one month of the CSF samplings were not included. There were only two patients who had been treated with azathiopirin since two months prior to study entry. No patients had been treated with other immunosuppressive drugs since these had effects lasting for many months. Those patients who showed only recurrent transverse myelopathy, recurrent optic neuritis, or recurrent brainstem symptoms were also excluded. Patients who were serologically positive for rheumatoid factor, anti-double stranded DNA antibody were also excluded. All of the patients were negative for anti-cardiolipin antibody (aCL) and anti-neutrophil cytoplasmic antibody (ANCA). Patients were screened for sarcoidosis with chest X-ray and detection of serum angiotensin converting enzyme. Patients, with the relapsing–remitting or secondary progressive course of MS were included in this study. As we aimed to examine the patients with conventional type of MS and as relapses were the main parameters in the present study, we excluded primary progressive MS patients.

2.1. Oligoclonal band testing

Concentration of IgG level in serum and CSF were determined by nephelometry. Both CSF and serum samples were diluted with distilled water to an IgG concentration of 25–30 mg IgG/L. A precast immune electrophoresis (IEF) gel (PhastGel™ IEF3-9, Pharmacia) expanded the pH gradient in the alkaline region with Pharmalyte 8-10,5 (Pharmacia). Pre-focusing procedure was started according to the program. After pre-focusing step at 50 Vh micro-pipette applicator containing 3 μL of sample was inserted into the anodic position of PhastGel. Samples with low concentrations of IgG level below 15 mg IgG/L were also analyzed with applying 6 μL unconcentrated CSF samples by using two sample applicators together. The separation was performed for a total of 510 Vh. After completion of the IEF run, detection of oligoclonal bands in the serum and CSF was performed by direct silver staining. Oligoclonal bands were defined as two or more distinct bands in the CSF sample but not visible in the paired serum sample.

For genetic evaluation human leukocyte antigen (HLA) typing was done.

2.2. HLA typing

2.2.1. Lymphocyte separation

Lymphocytes were obtained from peripheral whole blood by layering onto a Ficoll-Hypaque gradient to separate the blood cells by density gradient centrifugation. The separated blood lymphocytes were used for HLA-A, -B, and -C (class I) typing. The same lymphocytes were also used for the HLA-DR, and -DQ (class II) typing, after being enriched for B lymphocytes by magnetic separation with CD19-specific immunomagnetic beads (Lymphobeads HLA class II, Biotest, Germany).

2.2.2. HLA class I and class II typing

In parallel, a control group included 188 healthy subjects (104 female) who were homogeneous with the MS patients.

Typing of the HLA class I and class II antigens was performed by complement-dependent microcytotoxicity assays in 72-well Terasaki plates (HLA typing trays). Two commercial kits, Inno-Train Diagnostic and GTI, were used according to the manufacturers' instructions. All the HLA class I and class II trays were evaluated under ×200 magnification by fluorescent microscopic examination after staining with acridine orange and ethidium bromide (Fluoroquench, One-Lambda).

2.3. Procedure

Single HLA alleles were entered into the statistical analysis when their frequency exceeded 0.03 in either the patient or control groups, or when their relative frequencies compared with controls were ≤0.5 or >2.0. Eventually, five HLA alleles of the A locus (A1, A2, A3, A11,+//0A24), four of the B locus (B7, B8, B44, B51), and four of the DR locus (DR3, DR8, DR10, DR15) qualified for further analysis.

2.4. Analysis

Clinical variables between both groups with or without oligoclonal bands were analyzed with the non-parametric Mann–Whitney test. Analysis of percentages of different clinical signs at onset between the two groups was made with Fisher's exact test. The statistical analysis for HLA typing was performed by χ^2 test with Yates' correction to test the presence of an association. Fisher's exact test was used when the individual number was below five. p values were corrected for the number of comparisons (Pc), where necessary.

3. Results

Among the whole cohort of 210 patients, no CSF OB could be detected in 30 (14.3%) cases. Demographic and clinical data were in Table 1. 141 of the 156 female patients (90.4%) were positive for OCB, while 39 of the 54 male patients (72.2%) were positive. The female to male ratio was higher in the OCB-positive than in the OCB-negative group ($p=0.007$). Clinical course and disability, considered together with the disease duration from onset to lumbar puncture were similar in the two patient groups (Table 1). But mean relapse severity, e.g., the worst EDSS score at any relapse period was higher in patients without OCBs in CSF (Fig. 1). There was no relapse rate difference between the two groups in year 5. EDSS values got worse in OCB-negative group in year 5 ($p=0.008$). EDSS was also significantly higher in OCB-negative

Table 1
Demographic and clinical data of patients.

	OCB-positive	OCB-negative	p	Total
Patients n (%)	180 (85.7)	30 (14.3)		210 (100)
Female:male	3.61	1.0	0.007	2.89
Mean age	43.23 ± 11.40	45.03 ± 9.87	NS	44.34 ± 10.65
Mean age at disease onset	31.21 ± 9.38	30.98 ± 8.56	NS	31.13 ± 9.90
Mean relapse rate (biennial)	3.56	3.48	NS	3.52
Mean relapse severity (EDSS)	3.96	4.59	0.012	4.14
Mean EDSS at baseline	1.76	1.85	NS	1.80
EDSS score at year 5	2.01	3.12	0.008	2.33

Mean age: mean age at the lumbar puncture date, which was decided whether the patients are OCB-positive or OCB-negative.
Mean relapse rate: mean relapse rate in two years before the date of lumbar puncture, which was decided whether the patients are OCB-positive or OCB-negative.
Relapse severity: the worst EDSS score at any relapse period.
NS: not significant.

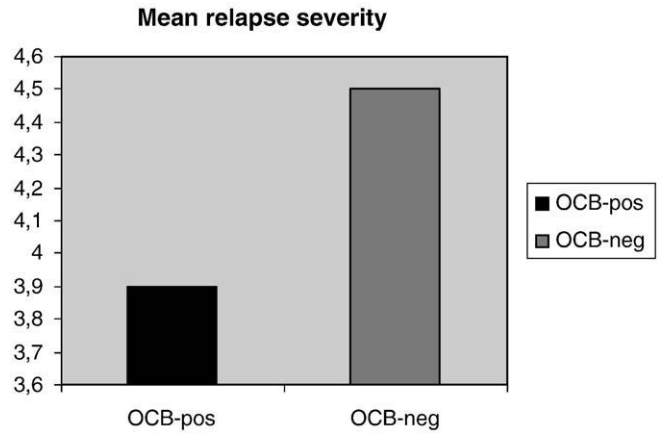

Fig. 1. Relapse severity in the study group.

group in year 5 ($p = 0.003$), which declared worse disability change in patients without OCB in CSF (Fig. 2). Mean EDSS score was also significantly higher in male patients compared to female patients (mean EDSS scores were 3.72 and 1.91, respectively. $p = 0.009$). There was no statistically significant difference regarding the requirement of DMT between the two groups. Percentages of DMT usage in OCB-positive and OCB-negative groups were 32% and 38%, respectively.

The frequencies of some HLA antigens were different among the OCB-negative, OCB-positive, and control patient groups, but most of these differences did not reach statistical significance after the p values were corrected. However, HLA DR15 antigen frequency was statistically higher in the OCB-positive than in the OCB-negative patients ($p = 0.007$) and control group ($p = 0.0002$). When comparing the two groups on the basis of disability on year 5, EDSS score was significantly higher in patients with HLA DR15 antigen (3.54 and 2.1, respectively. $p = 0.03$) (Table 2).

4. Discussion

As there are no specific markers of the disease, different clinical diagnostic criteria have been used for the diagnosis of MS. Presence of OCB in CSF is one of the important paraclinical tests for the diagnosis of MS (Andersson et al., 2006; Poser et al., 1983; McDonald et al., 2001). The presence of oligoclonal immunoglobulin G bands restricted to the CSF is the hallmark of the intrathecal humoral immune response and observed in a very high proportion of MS patients. Their presence can be demonstrated by isoelectric focusing (IEF) method;

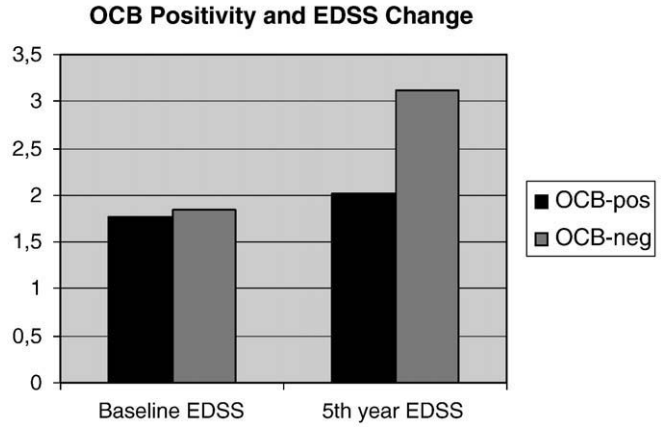

Fig. 2. EDSS values in 5 years follow-up.

Table 2
HLA gene frequencies in patients with OCB-positive, OCB-negative MS patients and healthy controls.

Gene	OCB-positive ($n = 180$)		OCB-negative ($n = 30$)			Healthy controls ($n = 188$)			
	N	GF	n	GF	p1	n	GF	p2	p3
A1	31	0.172	6	0.2	NS	35	0.186	NS	NS
A2	55	0.305	8	0.267	NS	49	0.260	NS	NS
A3	34	0.189	5	0.167	NS	37	0.196	NS	NS
A11	32	0.178	4	0.133	NS	35	0.186	NS	NS
A24	41	0.227	7	0.233	NS	45	0.239	NS	NS
B7	24	0.133	4	0.133	NS	18	0.095	NS	NS
B8	28	0.155	5	0.167	NS	11	0.058	0.003[a]	0.002[a]
B44	21	0.117	3	0.1	NS	18	0.095	NS	NS
B51	40	0.222	7	0.233	NS	44	0.234	NS	NS
DR3	24	0.133	4	0.133	NS	12	0.063	0.02[a]	0.02[a]
DR8	10	0.055	2	0.066	NS	9	0.047	NS	NS
DR10	21	0.117	3	0.1	NS	20	0.106	NS	NS
DR15	43	0.239	3	0.1	0.007[a]	10	0.053	0.000[a]	0.001[a]

GF, gene frequencies; NS, not significant.
p1, OCB-positive vs OCB-negative; p2, OCB-positive vs healthy controls; p3, OCB-negative vs healthy controls (all p values are corrected).
[a] Statistically significant.

which have been shown as the most sensitive method for the determination of OCB (Poser et al., 1983; McDonald et al., 2001; McLean et al., 1990; Lunding et al., 2000). With highly sensitive methods that allow the detection of IgG OCB in 95% of patients with MS, and if particular care is regularly taken to standardize the procedure for IgG OCB detection, the possibility of OCB-negative MS becomes rare (Link and Huang, 2006). There are transcultural, partly latitude-related differences in MS prevalence, and may be in the presence of OCB. A low prevalence has been reported from some Asian countries including Japan. Low frequencies of CSF OCB have been reported from Japan both in conventional MS and, in particular, in optic–spinal form of MS (OSMS) (Nakashima et al., 2006). The methods adopted for CSF OCB detection may play a role for low prevalence of CSF OCB. On the other hand, intra-CNS-related B-cells response may be uncommon in such patients. In the present study, there were 180 OCB-positive (85.6%) patients out of 210 MS patients. OCB detection was performed by isoelectric focusing. Our data is the first one from Turkey, where is believed to be placed between Western and Eastern world regarding the clinical and, especially immunological features. Although optimal methods for OCB detection were used, our data and others from the countries in southern Europe showed CSF OB less frequently compared to MS patients in i.e. Scandinavian countries or UK (McLean et al., 1990; Lunding et al., 2000; Link, 1973; Sa et al., 2005). Transcultural studies, comparing OCB presence are warranted to clarify the existence of such differences and their background, especially genetic features.

Whether OCB-negative MS patients have additional characteristic immune features remains to be explained. More recent studies have also reported lower rates of MS progression in patients without detectable CSF IgG OCB (Zeman et al., 1996a,b; Sa et al., 2005). Zeman et al. has suggested that OCB-negative clinically definite multiple sclerosis has a relatively benign prognosis. They found that patients without OCB were significantly less disabled than the matched OCB-positive patients (Zeman et al., 1996a,b). But there were only 12 OCB-negative patients when compared with 144 OCB-positive patients. Imrell et al. has suggested that OCB-positive and OCB-negative MS share the same clinical and demographic features with regard to female predominance and mean age at onset, in their study (Imrell et al., 2006). In a Japanese study, there was a female predominance in patients with OCBs, but no differences with regard to disease severity, disease course, or age at onset between OCB-positive and OCB-negative subgroups (Nakashima et al., 2002). In our study, we compared 30 OCB-negative patients with 210 OCB-positive patients. Female predominance was similar with Nakashima et al.'s (2002)

study. Mean relapse severity, EDSS in year 5, and EDSS change in 5 years were significantly higher in OCB-negative patients in our study. Despite its well-recognized disadvantages such as a poor cognitive decline and upper limb function, and a poor reproducibility, EDSS has been used as a primary outcome measure for neurological impairment and disability in clinical trials of MS, and it has been shown that EDSS might reflect to disability change for treatment effect (Ozakbas et al., 2004) and follow-up period (Ozakbas et al., 2005).

These results were unique, but before our study there was no prospective clinical design for comparison of OCB-positive and OCB-negative MS patients. Although follow-up period might be short, five year follow-up clearly demonstrated the difference between the two groups in the basis of prognosis, in the present study.

In a patient where CSF OCBs are expected but not detectable, the pair of CSF+-serum might be re-examined. but it has been suggested that the appearance of OB can sometimes be observed if lumbar puncture is repeated e.g. after 1/2–1 year or when new neurological manifestations. In our study, we performed lumbar puncture twice when we found negative results. Thus, when we judged as CSF OCB-negative practically it always remains OCB-negative.

Besides their clinical and demographic characteristics, the genotyping results of this study suggest that the two subgroups differ immunogenetically. The well-established association between HLA-DR15 and MS (Donmez et al., 2004) seems to be more prominent in OCB-positive MS patients, in our study. These results correspond well with those reported for Japanese patients with conventional MS (Kikuchi et al., 2003). Carriage of HLA-DR15 was also found in Swedish MS population (Imrell et al., 2006).

In conclusion, although determination of population with OCB-positive and OCB-negative MS constitutes distinct subpopulations, our results suggested that, MS patients with CSF OCBs have a female predominance, better clinical course with less disability and better prognosis, and are associated with HLA-DR15. These features reflect to “conventional” type of MS, which seems to be more favorable. Thus, we suggested that the more the patients have “conventional” MS, the better prognosis may be expected.

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