AN ESTIMATION OF OLIGOCLONAL BANDS IN PATIENTS WITH MULTIPLE SCLEROSIS

M. H. Harirchian[*1], A. Sarrafnejhad[2], M. Ghaffarpour[1] and H. Mojhdehipanah[1]

1) Iranian Center of Neurological Research, Department of Neurology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2) Department of Health, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Oligoclonal bands (OCB) can be detected in a significant percentage (90%) of patients with multiple sclerosis (MS), however, a much lower percentage has been reported in some studies, mainly from oriental countries. On the other hand, OCB have been detected in other neurological disorders (OND), even in diseases without any definite evidence for inflammation in CSF such as myasthenia gravis. This study was undertaken to evaluate the sensitivity and specificity of OCB detection in cerebrospinal fluid (CSF) in diagnosis of MS and to compare the clinical history of patients with and without OCB. CSF and serum of 80 patients, 40 with the diagnosis of MS and 40 with OND, were collected and analyzed for presence of OCB, using SDS-PAGE method. OCB were found in CSF in 28 (70%) of MS patients and in 8 (20%) of OND group. There was a significant statistical correlation between detection of OCB in CSF and MS diagnosis (P<0.0001). In MS group, the duration of the disease was longer in OCB positive patients (P= 0.045) and OCB detection was less common in patients with spastic paraparesis as a dominant clinical sign (P=0.006). In respect to other parameters, including age, sex and CSF cell count and protein, no significant difference was found. According to this study, there is a positive correlation between OCB and MS diagnosis as well as the duration of MS, and a negative correlation between OCB and spastic paraparesis as the dominant sign of MS.

Acta Medica Iranica, 42(4): 235-239; 2004

Key words: Multiple sclerosis, oligoclonal bands, cerebrospinal fluid, SDS-PAGE

INTRODUCTION

The ability to make an accurate diagnosis of multiple sclerosis (MS) and minimize the period of uncertainty for the patients is the major issue in the mind of today's neurologists. The diagnosis of MS rests on the demonstration of the white matter lesions disseminated in time and space, not explained by any other pathology.

While the history and clinical examination form the keystones of the clinical diagnosis, if these are not conclusive, many laboratory tests including magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis and evoked potentials can support the diagnosis. At present, after MRI, the most valuable and routinely performed test is assessment of IgG abnormalities in CSF.

In normal state, the CNS parenchymal tissue is clearly not a physiologic site for B lymphocytes aggregation and antibody production, but individual B cells can move across the blood-brain barrier, survive in the CNS and even produce substantial amount of immunoglobulins. In MS, small but consistent number of B lymphocyte, at different stages of differentiation, are demonstrable in inflammatory infiltrates in CNS, as well as in CSF. Indeed IgG abnormalities are the result of the activity of these cells. These abnormalities can be assessed

Received: 4 May 2003, Revised: ---, Accepted: 15 Oct. 2003

*** Corresponding Author:**
M. H. Harirchian, Iranian center of neurological research, Department of Neurology, Imam Khomeini Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 6948670, Fax: +98 21 6940033
E-mail: harirchn@sina.tums.ac.ir

quantitatively by IgG index and qualitatively by oligoclonal bands (OCB) detection.

OCB are two or more discrete bands in the gamma region that are absent or of lesser intensity in the serum. OCB detection can be done with various methods, including agarose gel electrophoresis (AGE), sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF).

In AGE method it is necessary to concentrate CSF about 50 times or more, and 3 to 5 ml of CSF is required for each test. In addition to large quantities of CSF that have to be obtained from patient, the procedure for the concentration of CSF may itself alter the electrophoretic pattern and obscure the separate bands. With this method OCB can be detected in up to 76% of MS patients (1).

SDS-PAGE is a method that can separates proteins according to their molecular weight. This method is more sensitive than AGE and can be used with very small volume of unconcentrated CSF, however, is technically more complex and difficult to perform. With this method, OCB has been reported in up to 94% of MS patients (1).

IEF is another method that afford superior resolution of closely migrating proteins or various forms of a single protein that differ in charge. This test is also done on unconcentrated CSF. At present, this is the most sensitive method that detects OCB in 90% (2) to 100% (3) of MS patients.

As mentioned above, with a sensitive method, OCB can be detected in CSF in a significant percentage (90%) of patients. However, in some investigations mainly from oriental countries, such as Japan, a much lower percentage (56%) has been reported (4).

Although OCB detection is a sensitive test, as can be expected, it is only an evidence of intrathecal inflammatory process and is not specific for diagnosis of MS or any other disease. OCB have been detected in CSF in diseases such as neurosyphilis, neurosarcoidosis, neuroborreliosis, subacute sclerosing panencephailitis (SSPE), encephalitis due to mumps, HIV, herpes simplex and herpes zoster, neurobehcet, Wegener granulomatosis, systemic lupus erythematosis, cerebral paraneoplastic syndrome, stiffman syndrome, and some primary and secondary tumors (2). OCB detection has also been reported in many other viral and bacterial disorders (5), Burkitt's lymphoma (6) and, unexpectedly, in diseases such as myasthenia gravis (7) and cerebral infarction (1, 8).

Many studies have been done to evaluate clinical and genetic differences betweens MS patients with and without OCB. In their study on Japanese MS patients, Fukazawa *et al.* found differences in HLA antigens between OCB positive and negative groups. According to their results, HLA-DR2 antigen, which has been confirmed to be associated with MS in many populations, was more common in the OCB positive than in the OCB negative and control groups. Furthermore, HLA-DR4 antigen was statistically more common in the OCB negative patients (4).

Pirttila and Nurmikko in their retrospective study on MS patients demonstrated that OCB negative patients were on average older, more often male, had experienced their first symptoms at a later age and suffered more often from primary-progressive form of the disease than OCB positive patients (9). In contrast, Sharief *et al.* and Gebraski *et al.* did not find any statistical correlation between age and OCB (10, 11). Although numerous studies have been performed in this regard in other countries, there has not been any systematic and validated study in our country. In this study we evaluated the frequency of OCB in CSF of patients with MS and other neurological disorders (OND), using SDS-PAGE method. We also analyzed the differences in parameters such as age, sex, duration of disease, clinical subtypes and predominant clinical sign in patients with and without OCB.

MATERIALS AND METHODS

We investigated serum and CSF of 80 randomly selected patients (40 with clinically definite MS and 40 with OND) admitted to the Imam Khomeini hospital from May 2001 to May 2003.

The mean age of MS patients at the time of study was 32.4 years (range 19-55). Poser's criteria were used for diagnosis of MS (12). Total number of patients with MS was 40 (female/male: 28/12). Of 40 MS patients, 32 had relapsing remitting (RR), 2 secondary progressive (SP) and 6 primary progressive (PP) subtypes. PP subtype was more frequent in older patients so that while proportion of

patients with PP subtype was 15%, in patients older than 40 years this raised to 57%. The duration of disease from its onset was 1-4 years in 22 patients, 4-8 years in 12 and more than 8 years in 6 patients.

The mean age of control group was 29.6 years (range 8-70) with female/male ratio of 22/18. In this group there were 10 patients with pseudotumor cerebri, 3 with dural venous sinus thrombosis, 6 with neurodegenerative diseases, 9 with peripheral neuropathy, 7 with infectious and noninfectious inflammatory CNS diseases, 2 with cerebrovascular disease, 2 with epileptic syndrome and 1 with myasthenia gravis. Lumbar puncture (LP) was done and CSF and blood samples collected simultaneously from all patients to detect the presence of OCB by SDS-PAGE method. CSF was also analyzed for cell count and protein. LP was a part of diagnostic evaluation in control group. In some cases such as myasthenic patient, LP was done to rule out other possible disorders. We obtained a written consent for LP from all patients. After collection of clinical and laboratory findings, analysis was done by Fisher exact and X^2 test, using SPSS software.

RESUTLS

OCB were found in 28 (70%) patients in MS group and 8 (20%) patients of control group, showing statistically significant difference (P<0.0001).

The youngest OCB positive patient was 19 and the oldest one was 55 years old. Age distribution of patients in relation to OCB is shown in table 1. There was not any statistically significant correlation between the age and presence of OCB in CSF (X^2 = 2.805; P= 0.094).

OCB were found in 75, 100 and 33.3 percent of RR, SP and PP subtypes, respectively. In spite of marked differences in percentages, there was not any statistically significant correlation between OCB and clinical subtype of MS.

OCB were detected in 14 of 22 patients with disease duration of 1-4 years, 8 of 12 with duration of 4-8 years and all of 6 patients with disease duration of more than 8 year. Statistical correlation between these variables was significant (P=0.045).

The relation of OCB to sex, CSF pleocytosis and proteins is shown in table 2. Seventy percent of patients were female, in whom OCB were found in 77.7%. In males, OCB were positive in 57%, however, correlations between these parameters were not significant (P= 0.498). Twenty five percent of patients had CSF pleocytosis and 15% had increased CSF protein. As shown in table 2, there was no statistically significant correlation between these variables and OCB.

Table 1. Age distribution of MS patients in relation to OCB

	OCB Positive		OCB Negative		Total	
Age (year)	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**
10-19	2	100	0	0	2	5
20-29	8	72.7	3	27.3	11	27.5
30- 39	15	75.0	5	25	20	50
40-49	2	50	2	50	4	10
50-59	1	33.3	2	66.7	3	4.5
> 59	0	0	0	0	0	0
Total	28	70	12	30	40	100

Abbreviations: MS, multiple sclerosis; OCB, oligoclonal bands.

Table 2. Clinical and laboratory data of MS patients in relation to OCB

Characteristic	**OCB Positive**	**OCB Negative**	***P* Value**
Female: male ratio	21:7	7:5	0.498*
CSF pleosytosis vs. normal CSF cells	8:20	2:10	0.693†
Increase CSF protein vs. normal CSF protein	6:22	0:12	0.153†

Abbreviations: MS, multiple sclerosis; OCB, oligoclonal bands; CSF, cerebrospinal fluid.

*X^2 test.

†Fisher's exact test.

Table 3. Dominant clinical syndromes of MS patients in relation to OCB

Clinical syndrome	OCB Positive		OCB Negative		Total		*P* Value
	N	Percent	N	Percent	N	Percent	
Spastic paraparesis	1	16.6	5	83.4	6	15	0.006*
Spastic quadriparesis	16	80	4	20	20	50	0.301*
Sensory sign	12	45	4	25	16	40	0.729*
Cerebellar sign	6	60	4	40	10	25	0.451*
Visual loss	13	65	7	35	20	50	0.730†
Diplopia	2	100	0	0	2	5	1.0*
Hemiparesis	1	100	0	0	1	2.5	1.0*

Abbreviations: MS, multiple sclerosis; OCB, oligoclonal bands.

*Fisher exact test..

†X^2 test .

In OCB positive patients, spastic quadriparesis was the most frequent sign (50%) and hemiparesis was the least one (2.5%). Table 3 shows dominant clinical sign of patients in relation to OCB. Of six patients with spastic paraparesis, OCB were found in only 1 patient (16.7%). There was a negative correlation between spastic paraparesis and presence of OCB in CSF, which was statistically significant (*P*=0.006).

In control group, OCB were found in one patient with SSPE, one patient with Guillain-Barre syndrome, one patient with neurobrucellosis, one patient with neurotuberculosis, one patient with venous sinus thrombosis with cortical infarction, one patient with myasthenia gravis and in 2 patients with undetermined diagnosis who showed inflammatory changes in MRI and CSF.

DISCUSSION

In this study OCB were detected by SDS-PAGE method in 70% of MS patients; excluding PP subtype, this rate would rise to 76.5%. Although it seems likely that the use of more sensitive methods (IEF) can raise these figures, it is still slightly lower than most other studies (2, 3, 9). Whether our patients had different HLA typing and immunogenecity remains to be defined. Nevertheless, there was significant correlation between OCB and MS (*P*<0.0001).

According to the study, in younger age group, the proportion of patients with OCB was greater than older ones, but as in Shariefs *et al.* and Gebarski *et al.* studies (10,11), there was not statistically significant correlation between age and OCB; however, such a relationship has been reported (9).

Disease duration in patients with OCB was significantly longer than patients without OCB, which is compatible with results of Pirttila *et al.* study (9) and could be related to greater likelihood of attacks in longer duration of disease.

In relation to sex distribution of patients, the proportion of female patients with positive OCB was greater than males, but in contrast to Pirttila *et al.* study, this difference was not statistically significant.

The concentration of CSF protein and pleocytosis in our patients was lower than most other studies, which have been reported to be increased in one third to one half of patients. These variables, like OCB, are measures of immune reaction, and the same reason that explains lower frequency of OCB may also explain these differences as well. Because pleocytosis has positive correlation with disease activity, another explaintion of lower amount of pleocytosis could be that some of these patients were not in acute phase of clinical attack.

As mentioned in results, OCB were not found in the majority of patients with spastic paraparesis. This finding could be explained by this fact that most of the patients in this group (4 out of 6) were belonged to PP subtype. In this subtype of MS the amount of positive OCB is lower than other varieties (9).

In control group, OCB were found in 20% of patients. The comparison of these findings with similar findings in other studies could not be accurate, because spectrum of disease in these studies was not similar. Although detection of OCB in

patients with myasthenia gravis and venous sinus thrombosis with cortical infarction was unexpected, such findings have been reported previously (1, 7, 8).

In conclusion, it appears likely that detection of OCB can be helpful in diagnosis of patients who are suspected to have MS but clinical or paraclinic findings are not diagnostic, especially with longer duration of symptoms and when primary progressive subtype of disease is not suspected.

REFERENCES

l. Iivanainen MV, Wallen W, Leon ME, Keski-Oja J, Calabrese VP, Krasny MA, Waybright EA, Selhorst JB, Harbison JW, Madden DL, Sever JL. Micromethod for detection of oligoclonal IgG in unconcentrated CSF by polyacrylamide gel electrophoresis. Arch Neurol. 1981; 38(7): 427-430.

2. McLean BN, Luxton RW, Thompson EJ. A study of immunoglobulin G in the cerebrospinal fluid of 1007 patients with suspected neurological disease using isoelectric focusing and the Log IgG-Index. A comparison and diagnostic applications. Brain. 1990 ;113 (Pt 5): 1269-1289.

3. Lunding J, Midgard R, Vedeler CA. Oligoclonal bands in cerebrospinal fluid: a comparative study of isoelectric focusing, agarose gel electrophoresis and IgG index. Acta Neurol Scand. 2000;102(5): 322-325.

4. Fukazawa T, Kikuchi S, Sasaki H, Hamada K, Hamada T, Miyasaka K, Tashiro K. The significance of oligoclonal bands in multiple sclerosis in Japan: relevance of immunogenetic backgrounds. J Neurol Sci. 1998; 158(2): 209-214.

5. Lolli F, Halawa I, Link H. Intrathecal synthesis of IgG, IgA, IgM and IgD in untreated multiple sclerosis and controls. Acta Neurol Scand. 1989; 80(3): 238-247.

6. Wallen WC, Biggar RJ, Levine PH, Iivanainen MV. Oligoclonal IgG in CSF of patients with African Burkitt's lymphoma. Arch Neurol. 1983;40(1): 11-13.

7. Adornato BT, Houff SA, Engel WK, Dalakas M, Madden DL, Sever JL. Abnormal immunoglobulin bands in cerebrospinal fluid in myasthenia gravis. Lancet. 1978; 2(8085): 367-368.

8. Rostrom B, Link B. Oligoclonal immunoglobulins in cerebrospinal fluid in acute cerebrovascular disease. Neurology. 1981;31(5): 590-596.

9. Pirttila T, Nurmikko T. CSF oligoclonal bands, MRI, and the diagnosis of multiple sclerosis. Acta Neurol Scand. 1995;92(6): 468-471.

l0. Sharief MK, Thompson EJ. The predictive value of intrathecal immunoglobulin synthesis and magnetic resonance imaging in acute isolated syndromes for subsequent development of multiple sclerosis. Ann Neurol. 1991;29(2): 147-151.

11. Gebarski SS, Gabrielsen TO, Gilman S, Knake JE, Latack JT, Aisen AM. The initial diagnosis of multiple sclerosis: clinical impact of magnetic resonance imaging. Ann Neurol. 1985;17(5): 469-474.

12. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol. 1983;13(3): 227-231.