Original Article

Follow-up of monosymptomatic presentation of multiple sclerosis according to immunological tests and clinical examinations

Alireza Vakilian[1,2], Gholamreza Bazmandegan[1,3], Mehran Ebrahimi[3], Abumoslem Rashidi Sharifabadi[3], Amir Moghadam Ahmadi[1,2*]

[1]Non-communicable diseases research center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, [2]Department of Neurology, School of medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, [3]Clinical research development center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, [4]Department of Epidemiology and Biostatistics, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

Correspondence: Amir Moghadam Ahmadi, Assistant Professor, Department of Neurology, School of medicine, Ali-Ibn Abi Talib Hospital, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, E_mail: clinical.research85@gmail.com

ABSTRACT

Background: Multiple sclerosis (MS) is one of the most common neurological disorders of the central nervous system leading to inflammation and destruction of the myelin sheath covering the nerve fibers of the brain and spinal cord. There is no definitive test for the diagnosis of MS. Differential diagnoses of MS include extensive inflammatory diseases, and autoimmune and collagen vascular disorders. To rule out the differential diagnoses of MS, many clinical, laboratory, and imaging tests may be used. The aim of this study was to evaluate the results of immunological tests in monosymptomatic patients for ruling out some of the differential diagnosis of MS. Methods: This was a descriptive study on patients who met the McDonald Criteria of monosymptomatic MS for evaluating immunological tests in them. Data obtained were analyzed using SPSS software version 20, and Chi-square and Fisher's exact tests. Results: The study consisted of 49 patients and controls. The mean age of the patients was 30.16 ± 9.12 years. In patients group, 11 (22.4%)men and 38 (77.6%)women were studied. The immunological tests were found to be positive in the range of 0 to 18% of examined patients in different tests. None of the performed tests changed significantly in our patients in comparison to control group. Conclusion: According to these results, we could confirm that serial clinical examinations are preferable to expensive immunological tests for the exclusion of the differential diagnosis of patients with MS.

Keywords: Multiple sclerosis, immunological tests, monosymptomatic presentation

Introduction

Multiple sclerosis (MS) is a chronic immune-mediated inflammatory disease of the central nervous system (CNS). It is characterized pathologically by perivascular infiltrates of mononuclear cells, demyelination, axonal loss, and gliosis with the formation of multiple plaques in the brain and spinal cord. Clinically, it is characterized by a variety of neurological signs and symptoms disseminated in time and space [1].

The diagnosis of MS is primarily clinical and relies on the demonstration of symptoms and signs attributable to white matter lesions that are disseminated in time and space, along with the exclusion of other conditions that may mimic MS [1].

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The list of disorders that could be confused with MS is very long and includes some items that are rather uncommon. The more important and more common disorders include collagen vascular diseases, autoimmune disease, abnormal blood vessels of the brain, CNS tumors, conditions that lead to degeneration of the nervous system, certain infections including Lyme disease, and even some psychiatric diagnoses [2]. Recently, a consensus approach on the differential diagnosis of MS described an effort to guide the clinical, laboratory, and imaging assessment of patients with a possible diagnosis of MS [3]. The distinct problem in the differential diagnosis of MS represents various laboratory tests that are often used as a screening tool for possible MS mimics. It has been suggested that screening patients with suspected MS with an unvarying battery of tests seldom generates a different diagnosis and more often leads to confusing false positive results. This is especially true of many tests ordered for the evaluation of systemic, inflammatory, autoimmune, and collagen vascular diseases [4,5].

The hypothesis that MS involves an autoimmune response to a self-antigen in genetically susceptible individuals induced by a previous unknown environmental-infectious agent evolved during the 20th century. This hypothesis was developed as a result of several discoveries, including the ability to induce an MS-like autoimmune disease in mammals through immunization with myelin or myelin antigens from the CNS [1].

Some autoimmune disorders include acute disseminated encephalomyelitis (ADEM), systemic lupus erythematosus (SLE), Sjogren's syndrome, anti- phospholipid syndrome (APS), and sarcoidosis [6-8].

Many autoantibodies have been identified in both serum and cerebrospinal fluid (CSF) of patients with MS, but unfortunately no autoantibody has been described as exclusively expressed in patients with MS compared to the respective fluids of healthy individuals [9]. Recent proteomic studies, on the other hand, have demonstrated that autoantibody in sera or CSF of patients with MS are reactive to a panel of proteins, rather than a single protein, suggesting a MS-specific pattern of autoantibodies [10]. Therefore, it is not surprising that many studies have shown present different autoantibodies that are used for the evaluation of systemic, inflammatory, autoimmune, and collagen vascular diseases in sera of patients with suspected MS [5]. Some of these autoantibodies include antinuclear antibodies (ANA), extractable nuclear antigen (ENA) profile, anti-neutrophil cytoplasmic antibodies (ANCA), anti-cardiolipin (aCL) IgG and IgM antibodies, C3, C4, CH50, anti-thyroid peroxidase antibodies (anti-TPO), anti-streptolysine titer (AST), and rheumatoid factor (RF).

One of the methods used to rule out differential diagnosis of this disease is immunological assays for the presence of auto-antibodies in patients with MS. The aim of this study was to evaluate a number of these immunological tests in order to exclude differential diagnoses of this illness such as inflammatory, autoimmune, or collagen vascular diseases and assess the value and usefulness of these tests in the diagnosis of this disease in patients with MS.

Methods

This was a descriptive study of patients consecutively referred to the MS clinic of Ali-ibn-Abi Talib Hospital in Rafsanjan, Iran, for evaluation of the results of immunological tests in patients with monosymptomatic presentation for a period of 5 years (2010-2014). All patients who met the McDonald Criteria for monosymptomatic presentation of MS were studied. This included all patients with one attack and one or more objective clinical lesions [11]. The number of patients who enrolled in this study and their immunological test results were gathered and evaluated was 49 individuals. Our control group consisted of 49 healthy person. The immunological tests that were performed on serum samples of patients with MS who entered the study included LE Cell, ANA, C3, C4, CH50, anti-cardiolipin antibody (aCL) IgM, aCLIgG, anti-phospholipid antibody (AntiPh) IgM, AntiPhIgG, ANCA, and RF tests. All tests were performed in the same laboratory using standardized methods suggested by the manufacturer. Anticardiolipin antibodies (IgM, IgG), ANA, p-ANCA, and c-ANCA were detected by enzyme-linked immunoabsorbent assay, Chorus kit. The cutoff values for anticardiolipin antibody IgM were lower than12 Mplu/ml negative, 12-18 Mplu/ml equivocal, and higher than 18 positive. The cutoff values for anticardiolipin antibody IgG were lower than 12 Gplu/ml negative, 12-18 Gplu/ml equivocal, and higher than 18 positive. The cutoff values for antiphospholipid antibody were lower than 12 AU/ml negative, 12-18 AU/ml equivocal, and higher than 18 positive. The cutoff values for ANA were lower than 0.8 U/ml negative, 0.8-1.2 U/ml equivocal, and higher than 1.2 positive. The cutoff values for p-ANCA and c-ANCA were lower than 12 AU/ml negative, 12-18 AU/ml equivocal, and higher than 18 positive.

Statistical analysis

After gathering the data, they were entered into the computer as special codes, and then, statistical analysis was performed using SPSS statistical software (version 20.0, SPSS Inc., Chicago, IL, USA) and chi-square and Fisher's exact tests. Chi-squared test, *t*-test, and fisher exact test were used to test the hypothesis.

Results

In this research, 53 patients were entered; four of them refused to follow the laboratory examinations, so the results of the tests on 49 patients with MS monosymptomatic presentation were studied. The mean ±SD age of the 49 patients who enrolled in the study was 30.16 ±9.12 years and their minimum and maximum ages were 16 and 52 years (most participants were aged 20-40 years), respectively. Moreover,11(22.4%) and 38 (77.6%) of MS cases were men and women. (Table 1) shows the prevalence of presentation symptoms and the participants sex and age. The mean ± SD of age of control group was 31.4 ± 7.6 ($P = 0.13$). 77.3% (37) of monosymptomatic cases and 80% (39) of control group were female. ($P = 0.81$)

In our research, all LE Cell tests of the 49 patients who were examined paraclinically were negative. ANA test was positive in 5 patients (1 man and 4 women). Of the total of 46 patients, only 6(13%) patients had positive results for C3 test (2 men and 4 women), and from among 44 patients, only 8 patients (18%) had positive results for C4 test (1 man and 7women). From 42 patients, only 3 CH50 tests results (7.1%) were positive (1 man and 2 women). The exact same result was obtained for aCLIgM test; 3 tests (6.1%) had positive results (1 man and 2 women) among 45 patients. In the case of aCL IgG test, a total of 47 patients tested, only 2 test results (4.3%) were positive, both were women. The exact same result was obtained for AntiPhIgM test, 2 test results (4.3%) were positive, both were women. Of the total 46 AntiPhIgG tests performed, the resultof none was positive. Of the 31 ANCA tests, only one case had a positive result; the case was a woman. From the 22 RF tests investigated, only one case result was positive that was a woman (Figure 1).

In control group RF and LE cell test was negative in all cases. ANA test was mildly positive in 2 cases, C3 and C4 test was mildly positive in 2 cases, CH50 was positive in one case. ACLIgM and IgG tests were negative in controls, AntiPh IgM and IgG tests were negative in controls, ANCA test was negative in these cases. The summary of data was shown in (Table 2).

MRI of these patients was compatible with MS according to McDonald Criteria 2010 [11]. After 2-5 years follow-up of our patients with various interferon therapy, all of them, except 3 patients, had new attacks and none of them presented other connective tissue disorders or autoimmune diseases signs and symptoms.

Discussion

MS is an autoimmune disease characterized by recurrent episodes of demyelination and axonal lesion mediated by CD4+ T cells with proinflammatory T helper (Th1 and Th17) phenotypes, macrophages, and soluble inflammatory mediators. The overactive proinflammatory Th1 cells and clonal expansion of B cells initiate an inflammatory cascade with several cellular and molecular immune components participating in MS

pathogenic mechanisms. In this scenario, autoantibodies and autoantigens have a significant role in immunopathogenesis, diagnosis, and therapeutic targets of MS [12, 13].

For a long time, myelin antigens were considered as the primary targets of the humoral autoimmune response in MS. However, recent studies have started to challenge this concept, as not only myelin, but nearly all CNS cells and even immune cells appear to be subject to autoantibody responses. Consequently, humoral autoimmunity in MS is much more variable and widespread throughout the brain than first thought. These autoantibodies have been detected against different CNS cell types, including neurons, oligodendrocytes, astrocytes, and even (infiltrating) immune cells. This variation in different autoantibody targets could be patient specific, and thereby, correspond to the heterogeneity in MS symptoms, disease course, and pathology [14].

As mentioned above, a large number of autoantibodies in serum and CSF of patients with MS have been identified, unfortunately, none of these autoantibodies are found specifically in patients with MS compared to normal individuals [10] Some recent studies have shown that autoantibodies in serum and CSF of patients with MS, instead of reacting with a certain protein, react with a group of proteins which can indicate specific autoantibody patterns in patients with MS [12]. That is why a large number of studies show a wide variety of autoantibodies that are used to evaluate systemic diseases, inflammation, and autoimmune and collagen vascular diseases in patients with MS and their presence is not unanticipated [5].

In this study, immunological tests results of 49 patients show that the LE Cell test was negative in all patients, ANA test was positive in 5 patients (10. 2%).In addition, 13% of patients showed change in C3 test (range 45-210mg/dl, normal range 90-180mg/dl), 18% of patients showed change in C4 test (range 7-63.37mg/dl, normal range 10-40 mg/dl). Moreover, ACL IgM and ACL IgG tests were positive in 7.9% and 4.3% of patients, respectively. All ACLIgM positive cases were in the range between 10 and 20MPLU/ml (weakly positive). Nevertheless, in the ACL IgG test, one case was observed in the highly positive range (5.40 U/ml) and one case in the moderately positive range (0.27 U/ml). In the anti-phospholipid IgM test, 5.3% of patients had positive results and all antiphospholipid IgG test results were negative. Only 4.5% of RF test results were positive. Only 3.2% of ANCA test results of patients (one case) were positive. It is noteworthy that, none of the patients who were examined, neither patients with positive nor negative test results, had any clinical manifestations except MS clinical manifestations in follow-up visits.

The prevalence of positive ANA test results in patients with MS, according to the other studies, varied from 2.5% to 80% [15]. In many previous studies, no association was observed between positive ANA test result and lupus symptoms, although a correlation was observed between ANA and MS disease activity by Collard et al. [14] According to the results obtained from our study, 10.2% of patients had positive ANA test results and none of them had clinical manifestations of lupus.

Furthermore, recent studies reported that the prevalence of positive results for anti-cardiolipin test (ACL) ranged between 4.8% and 44% [15]. For example, Heinzlef et al. reported that anti-cardiolipin antibodies were positive in 15% of patients with MS [16]. On the other hand, Rombos et al. did not report any higher prevalence of ACL antibodies (IgG or IgM) in patients with MS compared with the control group [17]. Our results show that ACLIgM and ACLIgG tests results were positive in 7.9% and 4.3% of patients, respectively. However, some studies have shown a higher rate of positive results for these tests in patients with MS [14]. Most of the published studies found no correlation between ACL and age, sex, disease duration, clinical classification, clinical evolution, or peculiar clinical symptoms [18]. In addition, they have not found any association between positive ANA test result and ACL IgM and ACL IgG test results.

In the current study, considerable changes in the level of C3 and C4 tests were observed in 13% and 18% of patients, respectively. Nevertheless, in previous studies, there was no difference between the mean of complement levels in patients with MS compared to normal individuals [19].

In the study by Etemadifar et al. performed on 79 patients with optic neuritis, results of p-ANCA and c-ANCA tests were not positive in case or control group participants [8]. In our study, only one ANCA test result (3.2%) was positive.

In agreement with previous studies, detection of RF was rare in our study (only one case). For example, in the research by Solomon et al. RF test result was positive merely in one case [7].

Contrary to the cohort study by Adamec et al. Which used the symptoms documented in patients' charts, in our study, patients were examined by a neurologist on a regular basis and regular follow-ups were performed in our MS clinic for 2-5 years [5].

According to our study, none of the mono symptomatic presentation our patients progressed to other disorders except MS and we can confirm that widespread immunologic tests without any main symptoms of these disorders were not useful or advisable.

Conclusion

According to the results, we can conclude that clinical examinations are preferable to the results of immunological tests for investigating the differential diagnosis of MS disease.

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Table 1: The first symptom of our patients

Symptom	Count	Mean age	Gender	
			Women	Men
Optic neuritis Diplopia	22	31.85 ± 10.7	18	4
Spastic paraplegia	10	33.6 ± 9.6	6	4
Sensory	6	28.16 ±5.9	6	0
Hemiparesis	6	27.83 ±7.5	5	1
Ataxia	2	30.25 ±4	1	1
Vertigo	2	29.5 ±1.5	1	1

Table 2: Test results

Test	Patients, n (%)	Controls, n (%)	P value
ANA[1]	5(10.2%)	2(4%)	0.218
RF[2]	1(2%)	0	0.50
ANCA[3] (p&c)	1(3%)	0	0.387
AclIgG[4]	2(4%)	0	0.199
AclIgM[5]	3(6%)	0	0.087
AntiphIgM[6]	2(4%)	0	0.087
AntiphIgG[7]	0	0	-
C3	6(13%)	2(4%)	0.077
C4	8(18%)	5(10%)	0.160
CH50	3(7%)	1(2%)	0.195
LEcell	0	0	-

1. Antinuclear antibodies, 2. rheumatoid factor, 3. antineutrophil cytoplasmic antibodies, 4 & 5. anticardiolipin IgG and IgM antibodies, 6 & 7. Antiphospholipid Antibody IgM &IgG

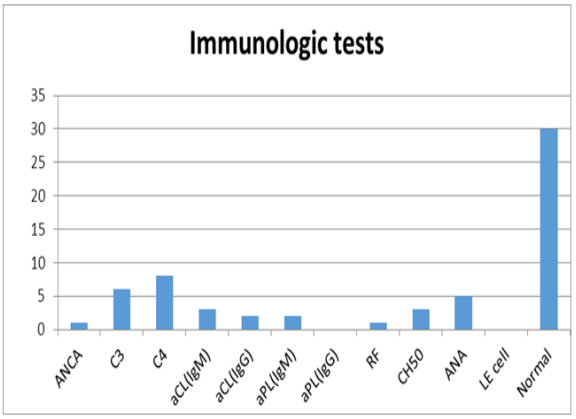

Figure 1: Immunological tests results in monosymptomatic patients