

# Evaluation of Serum Auto Antibodies in Multiple Sclerosis Patients: A Case Control Study

Sadegh Izadi<sup>1</sup>, Saeede Khoshniat<sup>2</sup>

<sup>1</sup> Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>2</sup> Shiraz University of Medical Sciences, Shiraz, Iran

## ABSTRACT

**Background and Purpose:** Multiple sclerosis (MS) is believed to be an immune-mediated disorder that develops from an interaction of the individual's genetic and as yet unidentified environmental causes. The prevalence of auto-antibodies in multiple sclerosis patients and their clinical associations vary in various studies. The aim of this study was to determine serum auto antibodies in multiple sclerosis patients.

**Methods:** This cross-sectional case-control study investigated anti-phospholipids antibody (APLA), antinuclear antibody (ANA), anti-cardiolipin antibody (ACLA), anti-neutrophilic antibodies (ANCA), anti-beta-2-glycoprotein I (anti  $\beta$ 2GPI), and anti-double strand DNA (anti-ds-DNA) in 54 consecutive patients with relapsing remitting multiple sclerosis (RRMS) and clinically isolated syndrome (CIS) who were referred to Imam Reza outpatient clinic of Shiraz University of Medical Sciences. The results were compared with 25 healthy individuals as the control group.

**Results:** Among 54 patients with relapsing-remitting MS or clinically isolated syndrome, at least one abnormal tests were found in 15 patients (27.9%), 6 (11.1%) had positive antinuclear antibodies, 3 (5.6%) had positive anti cardiolipin antibody (ACLA) and P-ANCA was positive in 2(3.7%) of patients and C-ANCA was positive in 1(1.9%) of patients. None of the patients had any clinical manifestations other than MS symptoms. In the patient group, anti-ds-DNA antibody was positive in 5.6% of cases; statistically it had no significant difference with the control group (0%) ( $P=0.7$ ), but anti- phospholipids antibody (APLA) and B2GPI were negative in all patient and control groups. The females had more positive auto-antibodies in comparison to males, but statistically their difference was not significant.

**Conclusion:** The results of this study showed that a significant number of patients with relapsing remitting multiple sclerosis and clinically isolated syndrome have positive serum auto-antibodies tests (including ANA, ANCA and ACLA) without clinical expression of any other autoimmune disease.

**Keywords:** multiple sclerosis, auto-antibodies, prevalence

ICNSJ 2017; 4 (1) :25-28

[www.journals.sbmu.ac.ir/neuroscience](http://www.journals.sbmu.ac.ir/neuroscience)

**Correspondence to:** Sadegh Izadi, MD, Clinical neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; Tel/ Fax: +98(711) 6121065; E-mail: [izadisad@sums.ac.ir](mailto:izadisad@sums.ac.ir)

**Received:** January 18, 2017

**Accepted:** February 27, 2017

## INTRODUCTION

Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system that affects mainly young and middle-aged people. There

are more than 2.5 million people with MS all over the world. Thus, MS is the most common demyelinating neuro-inflammatory disorder wherein the immune system destroys the myelin sheath of the axons for reasons that

remain unclear <sup>1</sup>.

A person with MS can have almost any neurological symptom or sign, which visual, motor, and sensory problems being the most common <sup>3</sup>.

The cause of MS is unknown; however, it is believed to occur as a result of some combination of genetic and environmental factors such as infectious agents <sup>3</sup>. Theories try to combine the data into likely explanations, but none has proved definitive. While there are a number of environmental risk factors and although some are partly modifiable, further research is needed to determine whether their elimination can prevent MS <sup>3</sup>.

The three main characteristics of MS are the formation of lesions in the central nervous system (also called plaques), inflammation, and the destruction of myelin sheaths of neurons. These features interact in a complex and not yet fully understood manner to produce the breakdown of nerve tissue and in turn the signs and symptoms of the disease <sup>3,4</sup>. Additionally, MS is believed to be an immune-mediated disorder that develops from an interaction of the individual's genetics and as yet unidentified environmental causes <sup>4</sup>. Damage is believed to be caused, at least in part, by attack on the nervous system by a person's own immune system <sup>3</sup>.

Multiple sclerosis is typically diagnosed based on the presenting signs and symptoms, in combination with supporting medical imaging and laboratory testing <sup>5</sup>. It can be difficult to confirm, especially early on, since the signs and symptoms may be similar to those of other medical problems <sup>3</sup>. The McDonald criteria, which focus on clinical, laboratory, and radiologic evidence of lesions at different times and in different areas, is the most commonly used method of diagnosis. with the Schumacher and Poser criteria being of mostly historical significance. While the above criteria allow for a non-invasive diagnosis, some state that the only definitive proof is an autopsy or biopsy where lesions typical of MS are detected <sup>3</sup>.

Several phenotypes (commonly named types), or patterns of progression, have been described. Phenotypes use the past course of the disease in an attempt to predict the future course. They are important not only for prognosis but also for treatment decisions. In 1996, the United States National Multiple Sclerosis Society described four clinical courses. The most common course is relapsing-remitting. This set of courses was later reviewed by an international panel in 2013, adding clinically isolated syndrome (CIS) and radiologically isolated syndrome (RIS) as phenotypes, but leaving the main structure untouched <sup>6</sup>.

Other autoimmune diseases including systemic lupus sclerosis and Wegener may mimic multiple sclerosis. The main aim of this study was to measure the level of serum autoimmune antibodies in patients with MS as an autoimmune disease of the central nervous system in comparison to the healthy control group.

## MATERIALS AND METHODS

In a cross-sectional case control study, the frequency of some circulating auto-antibodies including, anti-phospholipids antibody (APLA), antinuclear antibody (ANA), anti cardiolipin antibody (ACLA), anti-neutrophilic antibodies (ANCA), anti-beta-2-glycoprotein I (anti- $\beta$ 2GPI) and anti-double strand DNA (anti-ds-DNA) were measured in 54 consecutive patients with definite diagnosis of relapsing remitting multiple sclerosis (RRMS) or clinically isolated syndrome (CIS) who were referred to Imam Reza outpatient clinic of Shiraz University of Medical Sciences. The results were compared with 25 healthy individuals as the control group. Diagnosis of multiple sclerosis was based on 2010 McDonald criteria <sup>1</sup>.

This study was approved by ethical committee of Shiraz University of medical sciences.

## Statistical analysis

Statistical analysis to compare autoimmune antibody levels between patients and controls was performed by SPSS version 17 software. The Chi-square test was used for comparison of autoimmune antibody levels. P-values less than 0.05 were considered statistically significant.

## RESULTS

A total of 54 patients (88% female) with 25 healthy individual (87%female) matched for age and sex were enrolled in the study. The mean age of the patients was  $32.3 \pm 10.1$  years and that of the control group was  $29.4 \pm 6.1$  years. Among 54 patients with multiple sclerosis, abnormal tests were found in 15 patients (27.7%). Six (11.1%) MS patients showed presence of ANA positive test, with 4% positive test in the control group ( $p=0.037$ ). Anti-ds-DNA was positive in 3(5.6%) of MS patients without any positive test in the control group ( $p=0.041$ ). APLA and b2GP were negative in all cases (patients and controls). P-ANCA was positive in 2(3.7%) of patients and 4% of controls ( $p=0.09$ ) and C-ANCA was positive in 1(1.9%) of patients and 0% in controls ( $p=0.04$ ); ACLA was positive in 3(5.6%) patients and 0% of the controls ( $p=0.035$ ). No significant difference was found between males and females ( $p = 0.19$ ).

**Table 1.** Prevalence of serum auto-antibodies in patients with multiple sclerosis and healthy control group.

	ANA	Anti dsDNA	ACLA	APLA	P-ANCA	C-ANCA	Anti B2GP
RRMS	4 (13.3%)	2 (6.6%)	2 (6.6%)	0%	1 (3.3%)	1 (3.3%)	0%
CIS	2 (8.3%)	1 (4.1%)	1 (4.1%)	0%	1 (4.1%)	0%	0%
Total	6 (11.1%)	3 (5.6%)	3 (5.6%)	0%	2 (3.7%)	1 (1.9%)	0%
Control	1 (4%)	0%	0%	0%	1 (4%)	0%	0%
P-value	0.037	0.041	0.035	-	0.09	0.04	

RRMS= relapsing remitting multiple sclerosis, CIS= clinically isolated syndrome, APLA = anti-phospholipids antibody, ANA = antinuclear antibody, ACLA= anti cardiolipin antibody, ANCA=anti-neutrophilic antibodies, anti- B2GPI=anti-beta-2-glycoprotein I and anti-ds-DNA=anti-double strand DNA

Considering the type of multiple sclerosis, ANA was positive in 10% of RRMS and 9.1% of CIS; the difference was not statistically significant ( $p=0.31$ ). The results of other auto-antibodies in RRMS and CIS are shown in Table 1.

## DISCUSSION

Different circulating auto-antibodies like ANA, anti ds-DNA, ANCA, ACLA, APLA, which were seen in autoimmune diseases such as systemic lupus erythematosus and Wegener granulomatosis syndrome, were used for diagnosis of these autoimmune diseases<sup>6,7</sup>. These auto-antibodies may also be found in other non-related illnesses and in healthy individuals. A panel of these serum auto-antibodies is often obtained as part of the evaluation of patients suspected with having multiple sclerosis. Sometimes these auto-antibodies are seen in the patients with MS but the significance of these auto-antibodies in patients with MS is still unknown<sup>8-10</sup>. The prevalence of these auto-antibodies in multiple sclerosis (MS) patients differs significantly in various studies<sup>6,11,12</sup>. Our study showed that in a considerable number of the patients with multiple sclerosis (27.9%) at least one of these auto-antibodies was positive.

The frequency of antinuclear antibodies (ANA) in MS and their significance are uncertain. Previous studies have reported that ANA was found in 2.5% to 27% of patients with MS<sup>12-14</sup>. Most of these studies found no correlation between ANA and symptoms of SLE in MS patients<sup>12,13</sup>. Some cases of SLE may develop with MS-like clinical presentation and some of them may fulfill the criteria for both SLE and MS (lupoid sclerosis)<sup>12</sup>. Collared et al reported a correlation between the presence of antinuclear antibody and disease activity in patients with multiple sclerosis<sup>13</sup>. Also, a high level of ANA was reported in the patients with optico-spinal form of MS and neuromyelitis optica<sup>12</sup>. We have found positive ANA titers in 8.3% of CIS patients and 13.3 % of patients with RRMS. In our study, positive ds-DNA was found in 4.1% and 6.3% of CIS and RRMS patients, respectively. But none of them had symptoms of SLE. As our study

showed, some other studies have shown that there were no significant differences between ANA frequency in MS and control subjects<sup>11</sup>.

Some studies showed a prevalence of ACLA positivity in 4.8–44% of MS patients<sup>11,14</sup>. The prevalence of ACLA positivity in our study was 5.6 % (6.6% in RRMS patients and 4.1% in CIS patients). Miyagishi and his colleagues in their research reported two cases of multiple sclerosis with optic neuritis and positive ACLA<sup>15</sup>. Also, a case of multiple sclerosis with optic neuritis and ACLA positive was reported by Ogino et al<sup>16</sup>. In our patients, 44.4% of them had blurred vision and optic neuritis but in only one of them ACLA was positive.

The exact frequency of anti-phospholipids antibodies (APLA) in multiple sclerosis is unclear. IJdo et al in their research showed that a substantial number of APLA-positive patients had a concurrent diagnosis of MS or MS-like, sometimes presenting with optic neuritis, and transverse myelitis<sup>17</sup>. Also, Tatiana and his colleagues showed a significant APLA increase in MS patients compared to healthy controls, particularly during disease relapse which was associated with significantly higher values of anti-β2 glycoprotein<sup>9</sup>. In another research carried out by Cordoliani et al, it was shown that anti-phospholipids antibodies were found with a significant level in 8% of patients with MS<sup>18</sup>. In our study, APLA and B2GP were negative in all patients and controls. Patient selection method, study protocols, and sensitivity of techniques and inter-laboratory variation can be factors that reflect, at least partly, the variable reported frequencies of these auto antibodies in different studies.

## CONCLUSION

The results of this study showed that a significant number of patients with multiple sclerosis have positive serum auto-antibodies tests without clinical expression of any other autoimmune disease. It is possible that some patients with positive auto-antibodies developed clinical symptoms of other autoimmune diseases that declared itself subsequently. Therefore, further studies with long term follow up of patients are recommended.

## Acknowledgements

This study was supported financially by a research grant (Grant No. 3611) from Shiraz University of Medical Sciences and this article was extracted from thesis Saeede Khoshniat, medical student of SUMS. The authors would like to thank Dr. Nasrin Shokrpour for editorial assistance and Mrs Sareh Roosta for statistical analysis at Center for Development of Clinical Research of Nemazee Hospital.

## REFERENCES

- Goodin DS. The epidemiology of multiple sclerosis: insights to disease pathogenesis. *Handb Clin Neurol.* 2014;122:231-66.
- Marrie RA. “Environmental risk factors in multiple sclerosis aetiology”. *Lancet Neurol.* 2004;3 (12): 709–18.
- Weiner HL. Multiple sclerosis is an inflammatory T-cell-mediated autoimmune disease. *Arch Neurol.* 2004;61(10):1613-5.
- Compston A, Coles A. Multiple sclerosis. *Lancet.* 2008; 372:1502-17.
- Tsang BK, Macdonell R. “Multiple sclerosis- diagnosis, management and prognosis”. *Australian family physician.* 2011;40 (12):948–55.
- Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, Et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology.* 2014;83(3):278-86.
- Roach ES. Is multiple sclerosis an autoimmune disorder? *Arch Neurol.* 2004; 61:1615-16.
- Ramagopalan SV, Dymant DA, Valdar W, Herrera BM, Criscuoli M, Yee IM, et al. Autoimmune disease in families with multiple sclerosis: a population-based study. *Lancet Neurol.* 2007;6(7):604-10.
- Szmyrka-Kaczmarek M, Pokryszko-Dragan A, Pawlik B, Gruszka E, Korman L, Podemski R, et al. Antinuclear and antiphospholipid antibodies in patients with multiple sclerosis. *Lupus.* 2012; 21(4):412-20.
- Bonaci-Nikolic B, Jeremic I, Andrejevic S, Sefik-Bukilica M, Stojisavljevic N, Drulovic J. Anti-double stranded DNA and lupus syndrome induced by interferon-beta therapy in a patient with multiple sclerosis. *Lupus.* 2009; 18(1):78-80.
- Bitsch A, Dressel A, Meier K, Bogumil T, Deisenhammer F, Tumani H, et al. Autoantibody synthesis in primary progressive multiple sclerosis patients treated with interferon beta-1b. *J Neurol.* 2004; 251(12):1498-501.
- Koudriavtseva T, D’Agosto G, Mandoj C, Sperduti I, Cordiali-Fei P. High frequency of antiphospholipid antibodies in relapse of multiple sclerosis: a possible indicator of inflammatory–thrombotic processes. *Neurological.* 2014;35(11):1737-41.
- Garg N, Zivadinov R, Ramanathan M, Vasiliu I, Locke J, Watts K, et al. Clinical and MRI correlates of auto reactive antibodies in multiple sclerosis patients. *J Neuroimmunol.* 2007; 187(1-2):159-65.
- Wei Qiu, Bundell C, Wu JS, Castley A, James I, Hollingsworth P, et al. Low level of systemic autoimmunity in Western Australian multiple sclerosis patients. *Mult Scler.* 2010;16(3):351-4.
- Szmyrka-Kaczmarek M, Pokryszko-Dragan A, Pawlik B, Gruszka E, Korman L, Podemski R, et al. Antinuclear and antiphospholipid antibodies in patients with multiple sclerosis. *Lupus.* 2012; 21(4):412-20.
- Collard RC, Koehler RP, Mattson DH. Frequency and significance of antinuclear antibodies in multiple sclerosis. *Neurology.* 1997; 49: 857–861.
- Karussis D, Leker RR, Ashkenazi A, Abramsky O. A subgroup of multiple sclerosis patients with anticardiolipin antibodies and unusual clinical manifestations: do they represent a new nosological entity? *Ann Neurol.* 1998;44(4):629-34.
- Miyagishi R, Fukazawa T, Hamada T, Moriwaka F, Tashiro K. Two cases of recurrent optic neuritis (OPN) and acute transverse myelopathy (ATM) with associated anticardiolipin antibodies. *RinshoShinkeigaku.* 1992; 32(10):1121-4.