Anti –MBP autoantibody changes as a predictor of response to treatment in MS patients

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ABSTRACT

   Myelin basic protein (MBP) is one of the most important constituents of the CNS myelin sheaths. It is supposed that an autoimmune response directed against MBP is crucial in the demyelination process in patients with multiple sclerosis. Studies have proved that free anti-MBP level in CSF of MS patients is declined when the patient entered into clinical remission. Some researchers evaluate the changes in serum or CSF level of this antibody during immunomodulatory therapy; the results are different and the relation between the changes in this antibody and response to treatment is poorly investigated. The objective of this study was to assess the relation between the changes in serum level of anti-MBP and clinical remission in patients during treatment with fingolimod. 37 MS patients that were non responder to interferon and glatiramer acetate and were candidates to receive fingolimod were nominated for this study. In this study, the serum level of anti-MBP was evaluated before and after 3 and 6 months of therapy and clinical remission was assessed by changes in Expanded Disability Status Scale (EDSS) scores. The result of this study showed that MS patients, after treatment with interferon, have lower serum anti-MBP level than healthy control group and this difference is statistically significant ($p = 0.03$). The present study demonstrated that the serum anti-MBP level in MS patient during 6 months of treatment with fingolimod significantly decreased ($p < 0.001$). However, there was no significant difference in EDSS of MS patients during 6 months of treatment with fingolimod ($p < 0.001$).

Keywords: Multiple sclerosis (MS); Anti-MBP; Fingolimod

INTRODUCTION

   Multiple sclerosis (MS) is an inflammatory demyelinating disease that is considered as the most common disabling neurologic disease in young adults [1, 2]. In some ways, each person with multiple sclerosis lives with a different illness. Although nerve damage is always involved, the pattern is unique for each individual who has MS. While individual experiences with MS vary widely, doctors and researchers have identified several major types of MS. The categories are important because they help predict disease severity and response to treatment [3].

With regard to the development and progression of the disease, MS is divided into four types – MS; Relapsing-Remitting (RR) MS, Primary-Progressive (PP) MS, Secondary-Progressive (SP) MS, Progressive-Relapsing (PR) MS [4]. Autoimmune diseases such as MS involve the aberrant activation of T cells and/or B cells with epitope specificities that are usually excluded from the repertoire as the result of self-tolerance which is established during early development of the immune system [5].Thus, an increase in the autoimmune process could be interpreted as disease progression in multiple sclerosis [6].

Myelin basic protein (MBP) comprises about 30% of the central myelin protein and is limited on the cytoplasmic side of the myelin sheath [7]. MBP is an important autoantigen in multiple sclerosis, with the MBP (82–100) area being immune dominant for T cells and autoantibodies [8]. Because of co-localization of both B and T cell responses to the same MBP epitope, it is assumed that it plays an important role in the pathogenesis of MS [1]. The occurrence of antibodies against MBP is demonstrated in early multiple sclerosis [9]. B-cells, plasma cells and myelin-specific Abs are existent in chronic MS plaques and in areas of active myelin's breakdown in MS patients [10]. It has been previously reported that in the majority of patients, the MS inflammation had a specific autoimmune mechanism directed against MBP, specifically its immune dominant epitope P85 VVHFFKNIVTP96, which is located proximal to the tri-Proline sequence (residues 99, 100, 101) [11]. Several groups of investigators have evaluated the effect of some medicine on anti-MBP level in serum or CSF of MS patients [12, 13]. After being treated with presently approved immunomodulatory treatments, it takes at least 6–12 months for the clinical response to appear. Apparently, early assessment of response would be much better [14]. For this reason, there has been much interest in the development of applicable biomarkers as a predictor of the response to treatment. In this small pilot study, the possible relation between the immunological and clinical response to fingolimod treatment during 6 months interval was examined.

Materials and Methods
Patient selection

All patients who contributed in this project were seen at Sina MS research center in Tehran. Patients were diagnosed as having clinically definite MS by McDonald criteria confirmed by MRI of the brain. All patients had received interferon or glatiramer acetate for treatment but were volunteer for fingolimod at that time. The ethics protocol at Sina MS research center was followed. All patients were informed of the use of the blood that would be put prior to signed consent.

Healthy individuals who matched the participants of patient group in sex and age with no diagnosis of MS or other chronic diseases and with no MS patients in their family were registered in this project. Information about age, sex, and the clinical assessment of patients is shown in Table 1.

Table1: Clinical features of patients

Patient	Age (year)	Sex (male/female)	Disease duration (year)	EDSS
37	28.3±6.6	(8M/29F)	7.4±3.9	3±1.54

Notes: Data are expressed as mean ± SD. EDSS = Expanded Disability Status Scale; M = male; F = female.

Blood sampling

Venous blood was collected from MS patients into sampling tubes. Samples were centrifuged within 60 minutes after sampling at 1000× g for 15 min. Serum was then aliquoted and stored at −80˚C until assaying.

Anti -MBP determination

Serum samples were tested for levels of anti-MBP antibodies by enzyme-linked immunosorbent assay. For this assay, we purchased Human myelin basic protein autoantibody (MBP) ELISA Kit) Cat.No : SL1225Hu (from Sun Long Biotech Co., LTD. Briefly, this ELISA kit uses sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antigen specific to Human myelin basic protein autoantibody. Standard or sample are added to the appropriate Micro Elisa strip plate well and combined with the specific antigen.

After incubation and washing, a Horseradish Peroxidase (HRP) conjugated antigen specific for Human myelin basic protein autoantibody is added to each micro Elisa strip plate well and

incubated. Then free components are washed away and TMB substrate solution is added to each well. Only those well that contain Human myelin basic protein autoantibody and HRP conjugated Human myelin basic protein autoantibody Antibody would appear blue in color and turn yellow after the addition of the stop solution. The optical density (OD) and concentration was measured spectrophotometrically at the wavelength of 450 nm.

Clinical assessments

Quantitative neurological assessments and EDSS scores were assessed two times: before entry and after 6-month interval during treatment. An independent Neurologist, experienced in MS clinical trials, compiled the EDSS scores. Clinical progression was defined as a continuous (6 months) increase in EDSS scores of 1 unit if the baseline score was <5.5 or of 0.5 units if the baseline was 5.5 or higher.

Statistical analysis

The SPSS software (version 20) was used for the statistics analysis. Differences in base line anti- MBP level between MS patients and healthy subjects were tested by T-test. Comparisons between values obtained from the same patients during 6 months interval during treatment (baseline, after3 and 6 months) were performed by using the Repeated Measure test. Clinical progression was tested by one-sample T- test (all values were normally distributed). A p-value <0.05 was considered to be statistically significant.

RESULTS

In this study, the male to female ratio was similar in MS patients and the healthy control, and there were no significant difference (χ 2) regarding sex (p=0.5) and (T-test) age (p=0.75). All the patients were treated with beta interferons or with glatiramer acetate. During this study, five patients, for reasons such as drug side effects or non-cooperation were excluded.

Baseline levels of anti-MBP antibodies measured in MS patients vs. the healthy control

This study showed that MS patients, after treatment with interferon, had lower serum anti-MBP level (Figure 1) than healthy participants of the control group (p=0.03).

Levels of anti-MBP antibodies measured in the MS patients during treatment with fingolimod

Anti-MBP autoantibody levels in serum of most fingolimod treated patients were significantly (p<0.001) suppressed during 3 or 6 month. Figure 2 shows this suppression among these patients separated by sex.

Analysis of EDSS progressions at 6 months

Contingency analysis of the disease progression data at 6 months is shown in Table2. Patients are categorized in two groups (progressed and not progressed). Clinical progression considers sustained (6 months) increase in EDSS scores of 1 unit if the baseline score was <5.5 or of 0.5 units if the baseline was 5.5 or higher. There was not a statistical significant progression in the MS patients during 6 months of fingolimod treatment (Figure3)
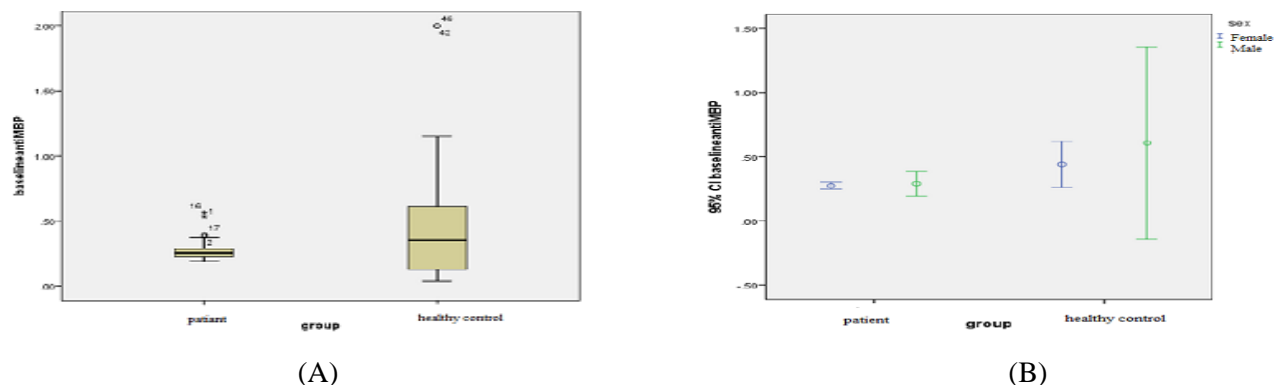

(A)　　　　　　　　　　　　(B)

Figure 1. The Levels of antibodies against MBP A) in MS patient and healthy group B) in two groups separated by sex
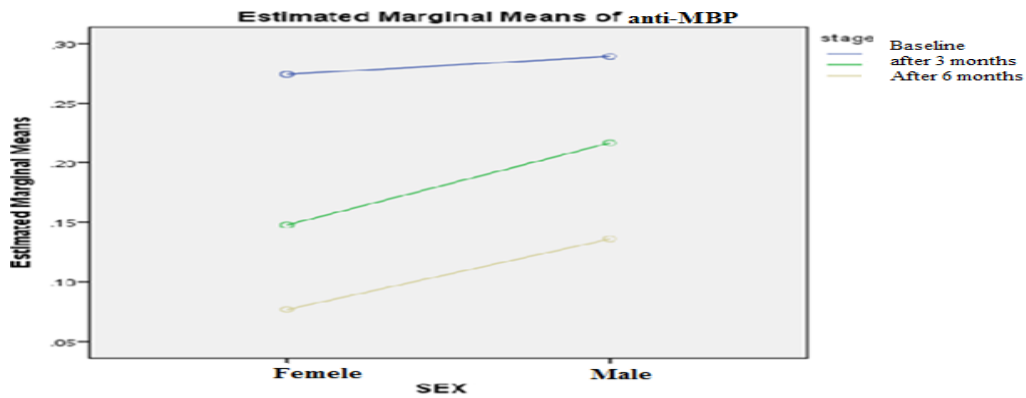
Figure 2. Anti-MBP suppression in fingolimod-treated patients

Table 2: Contingency analysis of the disease progression data at 6 months

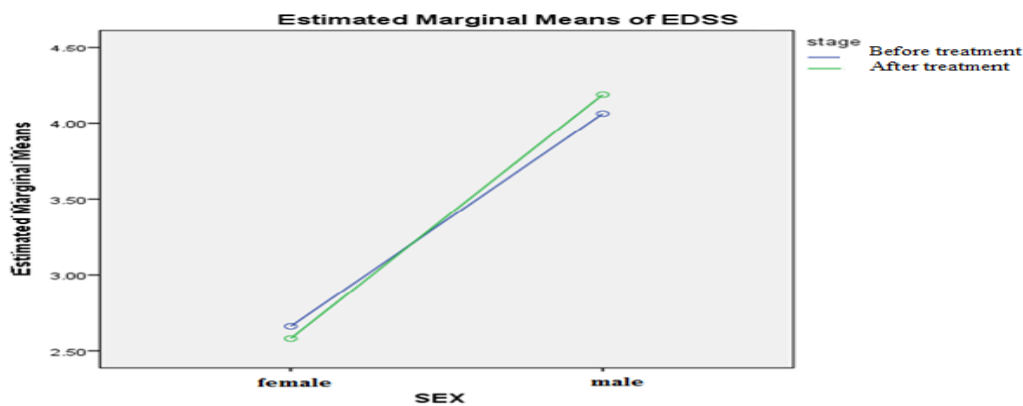| All patients n=32 , p< 0.001		
	female	**male**
Not progressed	24	7
Progressed	0	1
Total	24	8

Figure3. Estimated Marginal Mean of EDSS before and after treatment

This figure shows that women patients are more responder than men.

DISCUSSION

Demyelination in the brain and spinal cord is the main feature of the MS lesion. The tissue specificity points to an autoimmune response directed against the myelin sheath [15]. Several studies support the hypothesis that autoimmune mechanism plays a dominating role in the development of the multiple sclerosis. In the central nervous system, myelin basic protein (MBP) and proteolipid protein (PLP) are the main parts of the myelin protein [16]. MBP, through binding to negatively charged lipids on the cytosolic surface of oligodendrocyte membranes, is responsible for adhesion of these surfaces in the multilayered myelin sheath [17].Several techniques have been used to search for the presence of anti-MBP antigens in MS patients [16]. Evidence proposes that an increase in concentrations of Abs in CSF of these patients correlate with episodes of MS worsening [18]. Clinical benefits of IVIG and plasma exchange in MS have also implicated humoral mechanisms in the disease process [19]. Panitch et al. identified MBP autoantibodies in CSF in 81% of MS patients [20]. Warren and Catz had detected MBP autoantibodies in CSF of 90–95% of their patients with active disease and 77.7% of all MS patients [21].

The detection of autoantibodies in serum has been less successful. Cruz et al. detected MBP autoantibodies in CSF of 32% of their MS patients but were unable to detect autoantibodies in any of the matched serum samples using an immunoblot method. With the ELISA method, they detected MBP antibodies in CSF of 44% of their patients and 8% of the sera [22]. Based on these observations, anti-MBP may be involved in the pathogenesis of MS and therefore methods to prevent its synthesis and to keep it undetectable in the CNS are potentially important [1].

In this project, we have focused on the possible humoral aspect of immune system by looking for circulating antibodies against MBP in serum of MS patients. This study aimed at finding if anti-MBP level in serum of MS patient decreases during treatment with fingolimod and if there is any relationship between response to treatment and decrease in anti-MBP level. The results of this study are similar to data from previous long-term follow-up studies in some interesting ways.

First, in this study, it was shown that anti-MBP autoantibody levels in serum of most fingolimod treated patients were suppressed. Warren and Catz find this result in CSF of MS patient during treatment with MBP8298 (13). Second, antibody suppression was not predictive of clinical benefit. There was no significant difference in EDSS of MS patient during 6 months treatment with fingolimod. During this study, it was found that MS patients had lower serum anti-MBP level than healthy controls, after treatment with interferon. There is a controversy here. Angelucci et al. evaluated the effect of interferon-beta (β) therapy on anti-myelin antibodies. They concluded that 1-year of interferon-β treatment did not induce any changes in the levels of anti-MOG and anti-MBP antibodies. The present study suggested that in Iranian MS patient, interferon-beta (β) therapy may decrease serum anti-MBP antibodies level. These conflicting are: a) Analysis of antibodies intensely depends on the detection method used and its sensitivity and the conformation status of the antigen. For example, in solid-phase assays, the antigen is transferred to an artificial surface. As a result, protein denaturation may occur, and new epitopes not present in the native tertiary structure of the folded protein molecule may be exposed. b) It is imaginable that one antibody against a specific peptide sequence is directly concerned with myelin damage while the presence of antibodies against other regions of myelin protein may signify a secondary phenomenon not necessarily related to the disease.

In the other hand, it should also be noted that the duration of follow-up in this study was limited to 6 months. Obviously, follow-up for a long period would have allowed for detection of a correlation between antibody status and clinical benefit. Also a long-term study will be required to reliably define the role of HLA as markers of treatment response to fingolimod.

CONCLUSION

The results of this study show that there is anti-MBP in serum of healthy people, without any symptom of MS disease. As a result, the presence of antibodies against myelin antigens couldn't be the mere cause of MS and other conditions are needed for symptoms outbreak. However,

following the healthy people who have high serum anti-MBP level is effective for better understanding of this antibody's role in MS pathogenesis and may represents clear results in connection with this antibody and incidence of MS . Given the lower level of anti-MBP in MS patients than in healthy subjects after treatment with interferon, it is suggested that in Iranian MS patients, interferons may reduce this antibody's level. The baseline level of anti-MBP in one patient who has a significant increase in the EDSS level during these 6 months was higher than other patients. Perhaps Baseline blood serum anti-MBP levels can be used to identify populations of MS patients in which treatment with fingolimod is associated with a significant remission after 6 months of treatment with fingolimod.

ACKNOWLEDGMENTS

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