Original Article

Antibody profiling for the prognosis and diagnosis of multiple sclerosis in patients, compared with healthy subjects

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Abstract

Background: Multiple sclerosis is considered as an autoimmune disease of the central nervous system that is the main cause of disability in young adults around the world. The purpose of this study was to determine changes in antibodies in the prognosis of multiple sclerosis, and the use of antibody against aquaporin 4 for the diagnosis of multiple sclerosis. **Materials and Methods:** In this case - control study, 21 patients with a definite diagnosis of multiple sclerosis and 21 healthy subjects were selected as the study population. Blood and urine samples were collected, and nephelometry technique was used to assess the presence or absence of IgG, IgM and IgA in serum and urine samples. ELISA method for measuring of antibodies against aquaporin 4 was used. **Results:** There was no major difference in the mean of the total IgM in the case and control groups , but the mean IgA and IgG levels in the control group were evidently higher than in the case group. It was releaved that IgA, RBC and Hb mean differences between the two groups are statistically significant. Parallel with an increase in IgG, the probability of disease exacerbation was increased by 0.22, whereas with increasing ages, the probability of disease exacerbation was 15.0. There was also a positive and significant relationship between the average level of antibodies, IgG and IgM with the degree of illness However, the relationship between the mean serum IgA level and the degree of illness was inverse. It also became clear that antibodies against AQP-4 in serum and urine of patients with different degrees of illness showed no significant difference. The difference between the mean of antibodies against AQP in the serum of patients with mild and moderate MS was 54.1, but in mild and severe MS it was 53.3. **Conclusion:** The findings of this research suggest that serum antibody levels are directly related to the disease levels and can be used as a prognostic factor. Accordingly, it appears that the use of antibodies against aquaporin-4 in serum and urine for the diagnosis of this disease can be considered as a reliable approach.

Keywords: Multiple sclerosis, total antibodies, anti-aquaporin 4

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Introduction

Multiple Sclerosis (MS) is an inflammatory, recurrent and progressive disease of the central nervous system that leads to progressive neurological dysfunction. Due to the disability caused by this disease in young and aged people and also a growing global epidemic particularly in Iran, MS is considered important [1, 2]. The etiology of MS is unknown, but

some factors such as genetic and environmental factors, infectious agents and immunological factors are involved in the MS pathogenesis [3]. It is well known that the clinical course of MS in patients is unpredictable, and it varies in severity from mild to the progressive form [4, 5]. In this regard, the clinical course in MS patients is classified into 4 groups; relapsing- remitting, secondary progressive, primary progressive and progressive – relapsing [6] which are used to determine the prognosis and treatment of the disease. Studies in Iran suggest that in 64.4% of patients, the clinical course has been the relapsing - remitting type [7]. On the other hand, despite the determining role of T lymphocytes in the MS development, other studies suggest that antibody-dependent mechanisms are also involved in the immunopathogenesis of the disease [8]. Numerous studies have indicated the role of antibodies against various antigens in the diagnosis and prognosis of MS, like that of Markus Reindi, which disclosed the use of antibodies as important biomarkers in the determination and prognosis of MS [9]. Also, it has been reported an increase in IgG and IgM antibodies against neurofilament [10]. In another study it was shown an increase in anti-aquaporin 4 (anti-AQP-4) antibody in optical neuromyelitis, which is an autoimmune inflammatory disease similar to MS [11, 12]. Aquaporin 4 (AQP-4) is a water channel that leads to the passage of water across the cell membrane [13]. Additionally, many studies have shown that there a is relationship between production of antibodies in MS and the disease prognosis [14, 15]. Another study also showed that the increase in IgM antibodies in the cerebrospinal fluid may play a prognostic role in MS [16], whereas other studies did not find the relationship between antibody profile and MS prognosis [17, 18]. In summary, despite numerous studies regarding the changes in antibody levels in MS patients, the results of some studies were inconclusive, and most studies have been focused on the use of antibodies against various antigens while few are related to the anti-AQP-4 antibody prognostic role in MS prognosis [16- 20]. Hence, the aim of this study was to determine changes in antibodies in the prognosis of multiple sclerosis, and the use of antibody against AQP-4 for the diagnosis of MS.

Methods

In this case-control study, 21 patients with a definite diagnosis of MS disease and 21 healthy subjects were selected and a complete questionnaire on demographic information (age, sex, education, occupation), patient records, onset of symptoms, method of diagnosis, other diseases, disease progression, the classification of the onset of symptoms (sensory, motor, visual, etc.) was completed. Then, with the consent of the patient, 5 ml of blood sample and 5 ml of urine sample were taken. The presence or absence of IgG, IgM and IgA, in the total serum and urine samples was assessed by Nephelometry technique and Immunoglobulin measurement kit (binding sit). Nephelometer model was NOAD 2000 Mini nephelometr. Also anti-AQP-4 antibodies were measured by (ELISA Kit/Human/Aquaporin 4 Antibody) MyBioSource. Then the Optical density (ODs) of six standard solutions prepared with different concentrations (0, 3.0, 2.1, 5.2, 5 and 10 µl/ml) was measured in a wavelength of 450 nm using ELISA reader (2100 fax, Awareness Technology Inc Company). Standard curves were then drawn using the concentrations of the different solutions and the standard ODs. To determine the concentration of test samples, the OD of each sample was placed on the standard curve, and concentration estimation was performed.

Sample size calculations. The sample size, considering a test power of 90%, and a 5% error margin, with the variance δ^2=12 for IgG variable, and ε = 3.4 was calculated based on the formula below (21);

$$n_1 = n_2 = \frac{2(Z\alpha/2 + Z\beta)^2 \delta^2}{\varepsilon^2}$$

Statistical analysis. In the statistical analysis, the normality of the data was evaluated by Kolmogorov-Smirnov test using an SPSS 20 software package. Since the Kolmogorov - Smirnov test variable distribution of IgA antibodies in comparison with other antibodies exhibited a non-normal distribution, in order to compare the means of the variables; a non-parametric Mann-Whitney test was used. Results were expressed as the mean ± standard

deviation (SD) for each group and P≤0.05 was considered statistically significant.

Results

The results of the comparison of the demographic and laboratory variables, serum levels of IgG, IgM and IgA between case and control groups are presented in table 1.

As depicted in table 1, the average of WBC counts in the case and control groups was similar, but the average of RBC in the control group was significantly different compared with that in case group. Also, the difference between the averages of hemoglobin in the two groups was statistically significant. The amount of hematocrite in MS patients was lower than the average of hematocrit in the controls (data not shown) besides, no significant difference was observed in the number of platelets. Hematological index analysis such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) between the two groups had no significant difference

The results of the total serum antibody showed that there was no significant difference in the average of total IgM between the case and control groups, however difference of the average of total IgA and total IgG in these two groups was statistically significant. It should be noted that, the averages of these antibodies in the control group were considerably higher than in MS patients, albeit without statistical significance. Based on Mann-Whitney test we showed that only the mean difference of IgA, RBC and Hb was statistically significant between controls and patients. Also, the mean differences between IgA and RBC and Hb in the two groups were significant statistically but other variables had differences that were not statistically significant (Table 2).

In another test, logistic regression was used to assess the relationship between age and IgG, with severity of disease, by applying the formula:

$$\log\frac{p}{1-p} = -6.1 + 6.4x_1 + 0.199x_2 \quad \text{I}$$

Table 1: Demographic and laboratory variables, serum levels of IgG, IgM and IgA case and control groups.

		N	Mean	SD	P
Urinary AQP-4 Case (Without control) Iu/ml	case	16	17.38	2.1	-
Serum AQP-4 Case (Without control) Iu/ml	case	15	17.82	2.8	-
Age (years)	case	21	8.30	8.01	0.9
	control	21	31	15.22	
IgM (g/l)	case	21	1.52	0.93	0.6
	control	21	1.63	0.38	
IgG (g/l)	case	21	12.84	4.4	0.6
	control	21	13.38	2.4	
IgA (g/l)	case	21	2.50	2.05	0.7
	control	21	2.95	5.06	
Platelets ($/mm^3$)	case	21	2.44×10^3	90.8	0.7
	control	21	2.35×10^3	55.6	
RBC ($/mm^3$)	case	21	4.4×10^6	0.51	0.05
	control	21	4.7×10^6	0.56	
WBC ($/mm^3$)	case	20	7.04×10^3	2.78	0.9
	control	21	7.07×10^3	1.6	
Hb (g/dl)	case	20	12.38	1.5	0.05
	control	21	14.79	1.5	

*P≤0.05 was considered significant

Table 2: Comparison of the level of RBC/Hb/IgA between MS patients and controls based on Mann-Whitney test.

		Hb (g/dl)		RBC (mm^3)		IgA (g/l)	
Type	N	Average Rate	P	Average Rate	P	Average Rate	P
case	20	17.05	0.03	17.5	0.05	25.76	0.02
control	21	24.76		24.29		17.24	

Table 3: Logistic Regression showing the relation between IgG and age, with severity of disease.

	The coefficient of the regression line (B)	SE	P	OR
IgG(g/l)	0.199	0.122	0.105	1.22
Age(yeas)	0.142	0.079	0.07	1.15
invariant	-7.1	3.22	0.02	0.001

n this formula x_1 and x_2 is the age and IgG, and P represents the severity of disease, with each unit increase in IgG level, the probability of disease exacerbation is 0.22 and with every increase in age (years), the probability of disease exacerbation is 15.0 (Table 3).

Also, the average levels of antibodies against AQP-4 in serum and urine of control group compared with the severity of the disease (mild, moderate, severe), were not statistically significant. This implied that there wasn't much difference in the level of antibodies against AQP-4 in serum and urine of patients with different degrees of illness. Also a comparison between the levels of IgG, IgM and IgA in the case group in relation to the severity of the disease condition, showed no significant difference. Finally, the data of the difference between the average levels of IgG, IgM and IgA antibodies and antibody against AQP-4 serum according to severity of disease is summarized in (Table 4).

As shown in table 4, the difference in averages between patients with mild and medium MS is equal to 1.39, but in mild and severe MS it is equal to 5.93 and this is useful to analysis the severity of disease. We observed a relationship between the average IgG levels in the serum of MS patients and degree of the disease severity (Figure 1). As depicted in this figure, an increase in the degree of illness is associated with an increase in the average serum IgG level; which people with more severe disease have the highest level of serum IgG.

Table 4: Average difference in levels of IgG, IgM, IgA and Antibodies against AQP-4 in the serum based on the severity of the disease condition.

disease degree		average	average difference	P
IgG (g/dl)	mild moderate	1.397	11.52 12.92	0.73
	mild severe	5.93	11.52 17.46	0.07
IgA (g/dl)	mild moderate	2.11	1.84 3.96	0.05
	mild severe	-0.34	1.84 1.49	0.94
IgM (g/dl)	mild moderate	0.03	1.32 1.62	0.75
	mild severe	0.74	1.32 2.06	0.04
serum AQP-4	mild moderate	-1.54	17.86 16.32	0.97
	mild severe	3.53	17.86 17.82	0.92

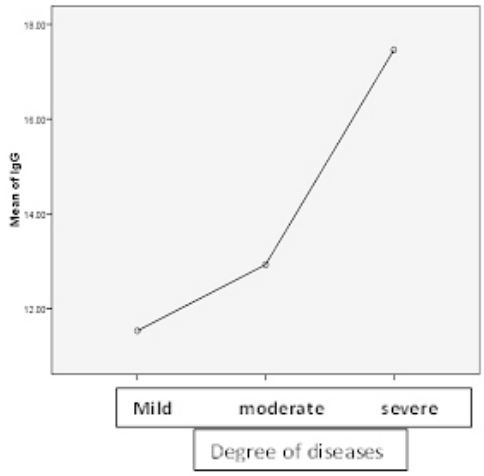

Figure 1. The relationship between serum IgG average levels in patients with MS and degree of disease severity.

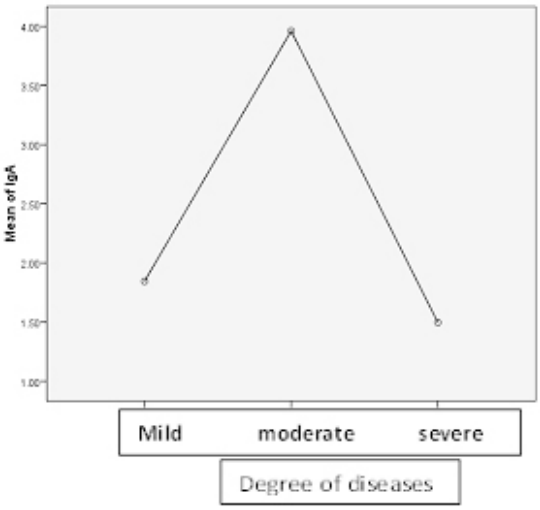

Figure 3. The relationship between average serum IgA in patients with MS and degree of disease.

Also similar relationship exists between serum IgM average levels in patients with MS and degree of disease but in an arithmetic progression, in such a way that the average IgM level in subjects with severe MS, is about double in comparison with in subjects with mild MS (Figure 2).

However, as shown in Table 4, the average of IgA level in moderate and mild groups, is significantly different from that observed for the mild and severe groups .The relationship is indirectly proportional between the average serum IgA levels and degree of disease, such that patients with a higher intensity of MS exhibit lower levels of serum IgA, whereas for patients having a mild form of the disease, the reverse is true (Figure 3).

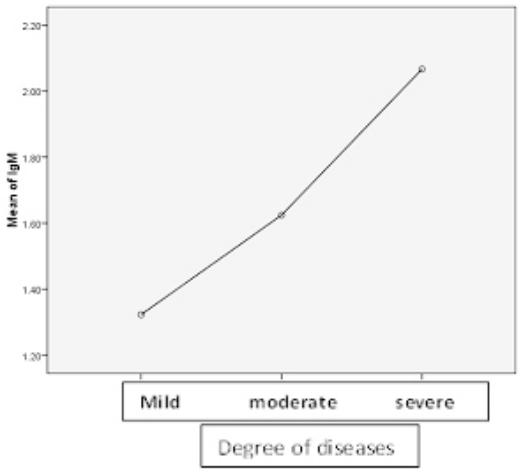

Figure 2. The relationship between serum IgM average level in patients with MS and degree of disease.

Also the average of antibody against AQP-4 in serum for patients with mild and moderate MS is -*1.54*, but that for patients with mild and severe MS is 3.53. Overall, it was determined that antibodies against AQP-4 in urine together with IgG, IgA, RBC, platelets, proteins, exhibited a negative correlation with age of the patients in the study group, and also had a direct correlation with other variables. Also the average of antibody against AQP-4 in serum for patients with mild and moderate MS is -*1.54*, but that for patients with mild and severe MS is 3.53. Overall, it was determined that antibodies against AQP-4 in urine together with IgG, IgA, RBC, platelets, proteins, exhibited a negative correlation with age of the patients in the study group, and also had a direct correlation with other variables. On the other hand, antibodies against AQP-4 in urine exhibited a direct correlation with platelets, IgM and IgG, while they had a direct correlation with the other variables like IgA, WBC, RBC, Hb, Hct, and patient age. On the other hand, antibodies against AQP-4 in urine exhibited a direct correlation with platelets, IgM and IgG, while they had a direct correlation with the other variables like IgA, WBC, RBC, Hb, Hct, and patient age.

Discussion

Different studies have been performed to assess the role of antibodies against multiple antigens in diagnosis and prognosis of MS. Anti-MOG antibodies as the

routine method for diagnosing MS disease, are appreciable but however, these antibodies can be seen in other nervous inflammatory diseases such as rheumatoid arthritis.

The present study assessed the level of antibodies in serum and urine of people with MS and their correlation with disease prognosis. We made a diagnosis based on the level of antibodies to AQP-4 in serum and urine samples of analyzed groups. In this study we assessed the level of total antibodies in serum and urine of MS patients and its relation to laboratory variables, and disease prognosis. At the same time based on the level of antibodies against AQP-4 in serum and urine samples of the patients the diagnosis of the disease were analyzed as well. In this regard, we observed a significant difference regarding hemoglobin in patients and healthy subjects in which the control group had higher levels of hemoglobin than in patients. Such changes in the hematocrit and MCV average were also particularly evident. In terms of the level of the variables expressed mentioned before it was more appropriate in controls. However, in the case of the patients, the level of the variables was reduced. In case of hemoglobin levels, the reduction was a significant indicator, since hemoglobin levels less than 11 g/dl can be considered as a signs of anemia (19). These results were consistent with those of Simpson et al. who compared laboratory indices in 15 patients with MS and 50 healthy subjects (20). Also in line with our study, Ross and colleagues mentioned the role of hemoglobin degradation products including met-hemoglobin which result to oxidation of sulfydryl groups in erythrocyte membrane, and finally damage the cell membrane in diseases such as MS, Rheumatoid Arthritis. Average of RBC in the control group was significantly higher in comparison with MS patients .Additionally, control group had a higher but non-significantly WBC count compared with MS patients. These findings are consistent with the results of Simpson et al. because they reported a higher average of leukocytes in the group of patients compared to a control group (21).

Our findings indicated that the increase of antibody levels in the serum of patients is directly related to increasing severity of disease. In addition, increased urinary and serum IgG antibodies against AQP-4 is an important factor in MS diagnosis. Several studies have described the role of antibodies against various antigens in the diagnosis and prognosis of MS.

Anti-MOG antibodies as a routine method for MS diagnosis is generally considered however, these antibodies with greater intensity can be seen in other inflammatory diseases of the nervous system, such as rheumatoid arthritis (9). Regression analysis showed for every unit increase in the mean total IgGthere is an increase in the severity of the disease with a rate of 0.2. These results are consistent with those of Shamon et al (19). In a study conducted by Ranks G. and colleagues on 115 MS patients and 92 healthy individuals, it was focused on the genetic and environmental influences on allergy and infection (22). Other studies have clarified the role of Ig in the pathogenesis of disease. Although the type and degree of illness was associated with total IgG and related subclasses, genetic polymorphism had no association with the disease (23). Also, IgM average in the case and control groups had no major difference. According to another study by Brett Schneider and colleagues, IgM level against the GAGA4 in patients with RRMS was greater, than in other diseases of the nervous system (24). The result of this study is consistent with our study, which indicates an increase in antibody is directly related to an increase in the severity of illness. In another study, the frequency of IgM against auto antigen such as phosphatidylcholine ethanol amine, neutrophil cytoplasmic, serine and phosphatidylinositol-nuclear antigen in MS patients with clinical illness have been reported more than others (25). In another study, serum IgM against antigens myelin oligodendrocyte glycoprotein and myelin basic protein has been reported to predict isolated syndrome-CIS conversion to MS disease (26). Although the role of antibodies as biochemical markers in predicting the disease recurrence or progression of MS is substantial, specific antibodies for this purpose have not yet been introduced (26). Perhaps some of the reasons regarding the differences in IgM level are the use of a non-specific antibody in the treatment and control groups, small sample size, and chronic nature of the disease. However, in our study, the mean total IgG as well as IgA in the control group was significantly higher than in the treatment group. Besides, the survey also showed that although the mean IgA in the control group was higher than in the

treatment group, the extent of disease in patients with severe MS is roughly twice that of patients with mild MS. This again confirms that antibody levels have a direct correlation with the severity of the disease. This finding also applies to the mean IgM and especially IgG, since the rate of increase for both types of antibodies in severe MS patients is far more than in the case of mild disease. The frequency of anti-AQP-4 IgG above normal range in patients was 80% for urine, and 94% for serum respectively, which indicated the high level of method sensitivity for detecting MS using serum and urine. Average antibodies in serum and urine samples had similar scores. Hence, the similarity between the average and the high frequency of positive reports confirms urine as having a diagnostic value roughly equivalent to serum levels. However, this result was not consistent with the results of Sei Hayat Kawa et al (27).

Considering the age of the patients, the mean age of patients with severe MS was six years higher than the average age in the mild group, indicating greater sensitivity of this test in our study. Thus we can assume that an average age of 36 years is related to disease severity, since disease progression is associated with antibody mean. In another perspective, the reduction of total antibodies in patients as compared with controls may be attributed to the antibody levels. Regression analysis on the level of IgG in relation to the severity of the disease indicates that a unit increase in the level of antibodies corresponds to a two fold increase in the severity of the disease.

Conclusion

The result of this study indicates that serum antibody level is associated with the degree of disease and can be used as a prognostic factor. There are equivalent numbers of antibodies against AQP-4 in serum and urine of MS patients, and sampling of serum and urine is reliable method for diagnosis. Urine sampling is not an invasive method, and it is easier to collect. Given the increasing prevalence of MS and the disabilities caused by MS especially in young patients, increase awareness and early diagnosis of the disease, particularly in clinical settings is important.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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