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RESEARCH ARTICLE

ASSESSMENT OF ANTI-MOG IN SERUM FROM PATIENTS OF MULTIPLE SCLEROSIS

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ABSTRACT

Myelin oligodendrocyte glycoprotein (MOG) is considered as a biokmaker in multiple sclerosis patients, MOG is unique to the brain, it is located on the outer lamellae of oligodendroglial membranes and myelin and it is highly immunogenic. **Objective:** In the present study an evaluation of serum anti-MOG antibody in relapsing remitting multiple sclerosis (RRMS) patients was carried out. **Method:** Serum levels of anti-MOG were measured by ELISA method after venous blood was collected from forty MS patients and forty healthy subjects as control group. Absorbance was read at a wave length of 450nm using ELISA reader. Anti-MOG antibody level was then calculated by plotting the optical density (O.D.) of each sample against the concentration in the standard curve. **Results:** A significant difference between serum Anti-MOG concentration in patients 554.85 ± 39.73pg/ml and the control group with 315.20 ± 28.49pg/ml, (p=0. 0211) was found. Also Anti-MOG has a negative correlation between age and concentration (r= -0.12) and significant positive correlation between expanded disability status scale (EDSS) and concentration (r= 0.28). **Conclusion:** Data for serum level of Anti-MOG Ab are present in this study. They are a basis for the role of Anti-MOG in multiple sclerosis patients. The present findings showed an increased levels of anti-MOG antibody in patient group which may suggest the demyelinating role of anti-MOG antibody and the importance of humoral immunity in relapsing remitting multiple sclerosis patients. Therefore the data found in this work might be used for further study on the importance of humoral immunity in autoimmune disease like MS.

Keywords: Multiple Sclerosis (MS), Myelin Oligodendrocyte Glycoprotein (MOG), Relapsing Remitting Multiple Sclerosis (RRMS), expanded disability status scale (EDSS).

INTRODUCTION

Multiple sclerosis (MS), the most common inflammatory disorder of the central nervous system (CNS), is characterized by an initial inflammatory phase followed by selective demyelination and finally neurodegeneration [1]. It is also characterized by recurrent neurological relapses and/or progression that occur within the white matter and cortical lesions within the central nervous system (CNS) [2].Multiple sclerosis lesions were infiltrated by T lymphocytes, macrophage, plasma cells, oligodendrocytes, astrocyte and axons[3]. Because of heterogeneity of this disease, identification of the subtype of patients by genetically, clinical and biological parameters will be necessary in future. Therefore the importance of identifying biological markers for MS has evolved over the past years [4]. The course of disease in multiple sclerosis is highly variable and hard to predict, ranging from a benign course to a classic relapsing–remitting, chronic progressive, or rare fulminant course [5]. Various antigens have been implicated as targets of the autoantibody response [6, 7]. Immunostaining studies have identified anti-MOG antibodies on disrupted myelin and within macrophages present in CNS lesions of MS patients. These auto antibodies were specifically bound to disintegrating myelin around axons in lesions of acute multiple sclerosis and the marmoset model of allergic encephalomyelitis [8].

The expression of MOG is confined to the CNS and sequestered at the outermost surface of the myelin sheath, this allow antibodies to access easily from the extracellular space [9]. The presence of plasma cells and myelin-specific Abs in both chronic MS plaques and acute MS lesions, strongly implicates their involvement in its pathogenesis. Immunoglobulin (Ig) G and complement deposition in MS tissues are typically found in areas of active myelin breakdown, suggesting its causative role [10].

An immunohistochemical study of lesion material of acute MS patients showed deposition of antibodies in fifty percent of MS patients, and it was suggested that the presence of antibodies in these lesions represents an immunopathological subtype of MS where antibodies are relevant [11]. If anti-myelin antibodies do contribute to demyelination in (a subtype of) MS, pathogenic antibodies could be a target for immune therapy, such as intravenous immunoglobulins (IVIg), which has shown beneficial effects in patients with relapsing remitting MS [12].

The aim of this study was to evaluate anti-MOG Abs serum level to clarify the role of these antibodies in MS disease and to find therelationship between the level of serum anti-MOG Abs and MS activity, diagnosis and clinical subtype.

MATERIALS AND METHODS

Sampling

The MS patients involved in this study were the patients attending the counsultaion clinic for Multiple Sclerosis, Baghdad teaching hospital in a 6 month period,(January 2012- June 2012).

The patients were carefully examined by a neurologist and a definitive diagnosis of MS was established based on MacDonald criteria concerning their clinical signs and symptoms and adjunctive diagnostic tools such as magnetic resonance imaging (MRI). Samples were collected from two groups: forty MS patients; twenty-three female and seventeen male and forty age and sex matched apparently healthy subjects considered as control.

All patients had relapsing-remitting MS and had not received any immunosuppressive therapy. Controls were persons without any history of autoimmune disease or other inflammatory diseases such as arthritis and colitis.

Informations were obtained from every patient participating in this study. Venous blood samples of patients and controls were collected and serum was separated and stored at -80 C.

Method

Antibody was measured using commercial ELISA (IBL, Germany) kits, in manufacturer's instructions applied to serum sample at room temperature (25 C), duplicate samples were applied.

Standard or sample was added to the wells that were coated with monoclonal antibody to human Anti-MOG Ab, then diluted biotin-antibody was added to each well and then diluted avidin(HRP) was added to each well, then TMB substrate solution was added to each well. Stop solution was added to all wells and absorbance was read at a wave length of 450nm using ELISA reader within 30 minutes.Anti-MOG antibody level were then calculated plotting the optical density (O.D.) of each sample against the concentration in the standard curve.

STATISTICAL ANALYSIS

Data were analyzed with descriptive statistics (mean ± SD), p<0.05 was considered statistically significant by using SAS. 2010. Version 9.1th ed.

RESULT AND DISCCUSION

Result

The group of relapsing-remitting multiple sclerosis (RR-MS) subjects in the relapsing phase (n=40) and an equal number of controls, the mean age of patients was 36.55±2.19 and in control was 36.55±2.19, and the mean EDSS of patients was 2.66 ± 0.91. The results revealed that the mean value of serum Anti-MOG antibody concentration in patients was 554.85pg/ml±39.73 and 315.20 pg/ml±28.49 in controls and the p-value was 0.0211. This means that there is a significant increase in serum Anti-MOG antibody concentration in patient compared to control (**Table1** & **Figure 1**)

Also Anti-MOG has a negative correlation between age and concentration (r= -0.12) and significant positive correlation between expanded disability status scale (EDSS) and concentration (r= 0.28) (Table 2).

Table1: Serum levels of Anti-MOG antibody (pg/ml) in MS patients and controls

Group(No.)	Mean ± SE Concentration (pg/ml)	Mean ± SE Age	Mean ± SE EDSS
Patients (40)	554.85 ± 39.73	36.55±2.19	2.66 ± 0.91
Control(40)	315.20 ± 28.49	36.55±2.19	
T-test value	138.74 *		
P-value	0.0211		

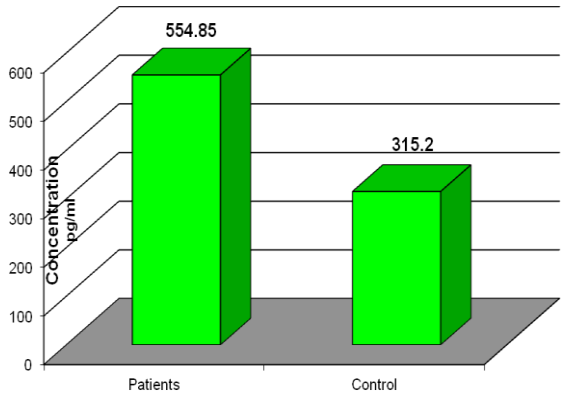

Figure 1. concentration of anti-MOG in serum of patients and controls

Table 2: Correlation coefficient (r) between anti-MOG concentration and both age and EDSS

Parameters	Variable	Correlation coefficient (r)
Anti-MOG Ab Conc.pg/ml	Age	-0.12 NS
	EDSS	0.28 *
* (P<0.05), ** (P<0.01), NS: no significant.		

EDSS = Expanded Disability Status Scale

Discussion

The results of this study which showed high serum levels of Anti-MOG antibody in MS patients compared to controls suggesting the role of anti-MOG Abs in demyelination and pathogenesis of MS disease.

Berger et al. showed that the occurrence of serum anti-MOG and to lesser extent antimyelin basic protein-specific IgM antibodies seemed to predict the incidence of new relapses in early MS patients [13]. Zhou et al. reported that the transduced cell lines were used to quantify antibody responses in serum. They found increased antibody reactivity to native MOG in MS patients compared with patients with other inflammatory disease of the CNS or healthy age matched control donors. According to the course of disease, all MS patient groups had higher antibody levels in serum compared with controls [14]. The number of patients with antibodies against native MOG was highest in primary progressive MS (PP-MS) patients [14].

Also antibodies against MOG cause demyelination in vitro [15] and in animal models of multiple sclerosis [16-17]. Mantegazza et al. examined serum and CSF reactivity of MOG by ELISA in a large cohort of MS patients with disease of varying severity and type. They found that mean serum levels of anti-MOG antibodies in MS and non-inflammatory -CNS patients were significantly higher than in healthy controls, while the frequency of positivity in patients with autoimmune peripheral nervous system (Aut PNS) disorders (Guillain-Barre syndrome) was similar to that of controls. These results indicate that anti-MOG antibodies are usually present only in patients with a CNS condition. Within the MS series, the secondary progressive subgroup had a significantly higher mean OD than the other MS subgroups [18]. Anti-MOG antibodies known to have demyelinating potential [15-16, 19] may be implicated in the degenerative changes (axonal and glial loss) characteristic of the secondary progressive form of the disease [20-22]. Mazzucco et al. found that MS patients and those with other neurological diseases, but not controls, had serum reactivity against a synthetic glycosylated MOG fragment but not against non glycosylated fragments [23].

Lassmann et al. found that the structure of the CNS lesions depended on the balance between encephalitogenic T cells and anti-MOG antibody. When EAE was induced with circulating anti-MOG antibody resulted in ubiquitous perivenous demyelination in the spinal cord and medulla oblongata [24].

Berger et al. showed that patients with a clinically isolated syndrome, the initial detection of serum antibodies against MOG and MBP predicts early conversion to clinically definite multiple sclerosis, whereas the absence of these antibodies suggests that the patient will remain disease-free for several years [13]. Patients who were seropositive for both anti- MOG and anti-MBP antibodies had clinically definite multiple sclerosis within a mean of 7.5 months. They found that the seropositivity for anti- MOG or both anti-MOG and anti-MBP antibodies, but not the number of lesions seen on MRI, was associated with an increased risk of relapse of multiple sclerosis. Thus, Berger et al. study demonstrates the importance of analysis of these antibodies, which is simple to perform and less expensive than MRI, can be used to predict the individual risk of the first relapse and therefore of clinically definite multiple sclerosis. So for patients who have seronegative antibody, may have a chance of remaining relapse-free for several years after the initial demyelinating event [25] and immunomodulatory therapy might be postponed until necessary [13].

The predictive value of this antimyelin antibodies may be important for counseling purposes or for early treatment to prevent the disease from getting worse [26, 25].

Mantegazza et al. also found a direct correlation between disability (EDSS score) and anti-MOG titer in progressive forms of the disease[18], which is in agreement with the present study which shows that there is a significant positive correlation between EDSS and anti-MOG concentration (r= 0.28), By contrast Karni et al. found no correlations between serum anti-MOG antibody levels and clinical parameters (disease course, EDSS score, and disease duration) [22].

Matsiota et al also showed that MS patients have higher levels of natural autoantibodies in their CSF compared to both healthy controls and patients with other neurological diseases [27]. Zhou et al. also identified serum antibodies against a strictly conformational epitope of MOG. The occurrence of antibodies with demyelinating properties further supports the pathogenic role of the humoral immune system in MS and calls for the development of B cell [28, 29].

In contrast Iglesias et al. shown that MOG-specific Abs and T cells are present in healthy controls as well as in MS patients [30].

Also previous studies supported the present study by showing that concentration of Anti-MOG Abs in MS patients is higher than healthy subjects [14, 18], and several papers have reported the presence of anti-MOG antibodies in the serum and cerebrospinal fluid (CSF) of MS patients [31-36], this immune response, when compared to a healthy control, was significantly elevated, implying an active antigen driven process.

The results of the present study showed the critical role of anti-MOG Abs in MS disease and the demyelinating potential of this biomarker and give a hope for early treatment to prevent the disease from progressing. Further studies on more MS populations and also, in vitro assessment of Anti-MOG Abs interaction are recommended to confirm the present results.

CONCLUSIONS

In this study of relapsing remitting multiple sclerosis patients have been identified and documented. MS appears to be a heterogeneous disease, with the presence of Ab-superimposed pattern. Inflammatory demyelinating diseases of the CNS includes MS could be better defined in the future according to the humoral response**.** The finding of specific and sensitive diagnostic parameters that allow the early diagnosis of MS will help to better define the most suitable therapeutic option or to develop new therapeutic agents**.** The study of auto-Abs is certainly an important way to better understanding the pathogenesis and to specify the different subtypes of CNS demyelinating diseases and to find an early therapeutic solution for the patients.

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