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Mannose-binding Lectin Mediated Complement Pathway in Autoimmune Neurological Disorders

Mehrdad Farrokhi[1], Mehrnoosh Dabirzadeh[2], Nastaran Dastravan[3], Masoud Etemadifar[4], Keyvan Ghadimi[2], Zahra Saadatpour[5], and Ali Rezaei[6]

[1] Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2] Department of Neurology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[3] Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
[4] Multiple Sclerosis and Neuroimmunology Research Center, Isfahan, Iran
[5] Department of Radiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[6] Department of Radiology, School of Medicine, Najafabad Branch, Islamic Azad University, Isfahan, Iran

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ABSTRACT

Multiple sclerosis (MS) is a complex, demyelinating disease of the central nervous system (CNS) with variable phenotypic presentations, while Guillain-Barre Syndrome (GBS) is the prototypic acute inflammatory disorder that affects the peripheral nervous system. Myasthenia gravis (MG) is a T cell dependent and antibody mediated autoimmune disease. Although it has been shown that complement plays a critical role in the pathogenesis of MS, GBS, and MG, the role of mannose-binding lectin (MBL) as a biomarker of immunopathogensis of these diseases and also its association with the severity of them have been poorly investigated. Therefore, in this study we aimed to measure plasma levels of MBL in patients with MS, GBS, and MG.

In a case-control study, plasma was obtained from healthy controls (n=100) and also patients with MS (n=120), GBS (n=30), and MG (n=30). Plasma level measurement of MBL was performed using enzyme-linked immunosorbent assay (ELISA).

The mean serum level of MBL was significantly different between groups of patients and healthy controls (p<0.001). We also found a positive correlation between plasma levels of MBL and severity scores of MS, MG, and GBS patients including: expanded disability status scale (EDSS) (r=+0.60 and p=<0.001), quantitative myasthenia gravis score (QMGS) (r=+0.56 and p=0.01), and GBS disability scale (GDS) (r=+0.37 and p=0.04).

Taken together, our findings suggest that complement activation mediated by MBL contributes to the pathogenesis and also severity of MS, MG, and GBS. However, because the lectin pathway can be involved in several phases of the immune response, further evidence will be required to elucidate the underlying mechanism.

Keywords: Autoimmunity; Complement system proteins; Guillain-Barre syndrome; Mannose-binding lectin; Multiple sclerosis; Myasthenia gravis

Corresponding Author: Mehrdad Farrokhi, MD; Immunology Department, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: (+98 93) 3781 3965, Fax: (+98 31) 3627 3910, E-mail: mehrdadfarokhi72@yahoo.com

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INTRODUCTION

Multiple sclerosis (MS) is a complex, demyelinating disease of the central nervous system (CNS) with a variable phenotypic presentation and subsequent disease course that cannot be predicted at disease onset. Although there is still no generally accepted model for the cause of MS, pathogenesis involves multiple inflammatory and apoptotic processes in the CNS which contributes to its characteristically variable pathology, phenotypic presentation, disease course, and outcome.[1,2] Several potential biomarkers such as cytokines, chemokines, and cell surface antigens have been suggested as aids for determining disease course, prediction of relapses, the early initiation of therapeutic interventions, and response to treatments in MS.[3,4] However, their clinical usefulness has not yet been clearly established because their measurement is time consuming and the sensitivity of the measurement methods is insufficient. Complement system is part of the innate immune system, but it can be recruited into action by the adaptive immune system, especially in defense against infections, immune complex diseases, and neurodegenerative disorders.[5] Although the role of alternative pathway in the pathogenesis of MS has been documented in many studies, there is still a great deal of uncertainty about the roles of other pathways of complement system in the disease.[6] It has been shown that complement system can cause demyelination in MS patients directly by inducing lysis of oligodendrocytes and also chemoattraction of macrophages into the site of inflammation, however, complement mediated astrocyte damage has been suggested as the initial event in CNS inflammation in MS patients.[7,8] Results obtained from different studies investigating the phase of complement activation in MS patients have been often conflicting.[9] Although the immunopathogensis of MS, including the breakdown of self-tolerance and the consequence occurrence, is mostly attributed to adaptive immune system dysfunction, innate immune system may also play a crucial role in the pathogenesis of MS.[10] It has been shown that mannose binding lectin (MBL) is primarily produced in the liver and is distributed throughout the body, but within the CNS the majority of complement components are synthesized by glial cells and neurons; and their production is increased in response to inflammatory stimulators. In MS, numerous studies have indicated the significant role of complement in the pathology of MS and in this regard many complement components have been measured in sera and CSF of MS patients as potential biomarkers of disease activity.[6] However, the majority of these biomarkers were limited to measurement of C3, C4, C5, and terminal component complex, which led to the conflicting results, therefore the exact immunological role of complement in MS pathogenesis remains poorly understood.[11,12] Guillain-Barre Syndrome (GBS) is the prototypic acute inflammatory disorder and the foremost cause of post-infectious neuromuscular paralysis worldwide that consists of several pathophysiologically distinct subtypes.[13] The worldwide overall incidence of GBS is estimated to be 1.1-1.8 per 100,000, with acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is the most common underlying subtype.[14] It has been demonstrated in studies both in patients and animal models that activation of the complement system by antibodies against peripheral nerve myelin may have a pivotal role in the pathogenesis of the GBS including damage inflicted upon the myelin sheath in AIDP and axolemma of motor fibers in acute motor axonal neuropathy (AMAN).[15-17] Myasthenia gravis (MG) is a T cell dependent and antibody mediated autoimmune disease, characterized by fluctuating muscle weakness induced by widespread neuromuscular junction (NMJ) damage. Anti-acetylcholine receptor (AChR) antibodies as the main pathogenic factors in MG pathogenesis are present in sera from 80% to 90% of patients with generalized MG.[18] It has been shown that AChR antibodies bind complement and orchestrate the complement cascade producing a complement-mediated impairment of the neuromuscular junction which leads to the reduction of AChRs.[19] Although it has been shown that complement plays a critical role in the pathogenesis of GBS and MG, the role of MBL as a biomarker of immunopathogensis of these diseases and also its association with severity of them have been poorly investigated.

Therefore, in this study we aimed to measure serum levels of MBL in patients with autoimmune neurological disorders including: MS, GBS, and MG as well as the correlation between the mean serum concentration of MBL with severity and other clinical characteristics of patients.

MATERIALS AND METHODS

In this case control study, 120 relapsing remitting multiple sclerosis (RRMS) patients (Mean age: 37.17±9.14), 30 MG patients (Mean age: 38.20±5.06), 30 GBS patients (Mean age: 36.60±10.14) from Alzahra hospital of Isfahan University of Medical Sciences and also 100 age and sex matched healthy subjects (Mean age: 36.12±8.65) as control group from Blood Transfusion Organization of Alzahra hospital were included. All of the patients fulfilled their diagnosis according to the related criteria.[20-22] Patients were in the remission phase of the disease and none of them were under treatment within the last month prior to the study. Exclusion criteria included other autoimmune disease, blood systemic disease, inflammatory disease, and immunosuppressive therapy. The severity of patients with GBS, MG, and MS was evaluated by two independent neurologists using the GBS disability scale scores (GDSs), the quantitative myasthenia gravis score (QMGS), and expanded disability status scale (EDSS), respectively. The protocol of this study was reviewed and approved by the Medical Ethnical Committee of Isfahan University of Medical Sciences. Written informed consents were achieved from all the subjects who participated in this study.

Peripheral blood samples were collected from three groups of patients and also healthy controls. Plasma content was recruited and stored at -80ºC. Plasma level measurement of MBL was performed using enzyme-linked immunosorbent assay (ELISA) commercial kits (Hycult Biotechnology, Uden, the Netherlands) according to the manufacturer's instructions. Serum MBL concentrations were expressed as ng/ml.

The normal distribution of MBL plasma levels of subjects was assessed using Kolmogorov-Smirnov Z-test. One-way ANOVA test was used for comparison of plasma levels of MBL between groups of cases and healthy controls. Pearson correlation test was done in order to evaluate correlation between plasma levels of MBL and severity of GBS, MG, and MS. Statistical analysis was performed using SPSS (ver. 19). Data were shown as mean±SD and number (percent). P-value less than 0.05 was considered as significant threshold.

RESULTS

There were no statistically significant differences between cases and healthy controls regarding age and sex; three groups of patients and healthy controls were matched well regarding age and sex (p=0.75 and p=0.93, respectively). Other clinical and demographic characteristics of patients and healthy subjects are summarized in table 1.

The mean serum level of MBL was significantly different between groups of patients and healthy controls (p<0.001). The serum levels of MBL were significantly higher in GBS patients compared to healthy controls and MS patients (p<0.01). The serum levels of MBL were also higher in MG patients compared to RRMS patients and healthy controls (p<0.01). The mean serum level of MBL in RRMS patients was significantly higher than healthy controls (p<0.01). Our analysis did not show any significant difference between MG and GBS patients regarding serum levels of MBL (p=0.98) (Figure 1).

We also found a positive correlation between plasma levels of MBL and severity scores of MS, MG, and GBS patients including: EDSS (r=+0.60 and p<0.01), QMGS (r=+0.56 and p=0.01), and GDS (r=+0.37 and p=0.04) (Figure 2).

Table 1. Clinical and paraclinical features of healthy controls and patients with MS, GBS, and MG

Characteristics	RRMS	GBS	MG	Healthy control	*p*-value
Number of subjects	120	30	30	100	-
Gender M/F	40/80	11/19	9/21	36/64	0.91
Age (Mean±SD)	37.17±9.14	36.60±10.14	38.20±5.06	36.12±8.65	0.28
EDSS (Mean±SD)	2.61±1.43	-	-	-	-
GDS (Mean±SD)	-	2.93±1.22	-	-	-
QMGS (Mean±SD)	-	-	13.63±5.85	-	-
MBL (Mean±SD)	1556.4±460.8	2449.4±471.4	2404.2±494.1	1109.5±527.6	0.00

RRMS: Relapsing remitting multiple sclerosis, GBS: Guillain-Barr'e syndrome, MG: Myasthenia gravis, EDSS: Expanded disability status scale, GDS: GBS disability scale, QMGS: Quantitative myasthenia gravis score, MBL: Mannose-binding lectin.

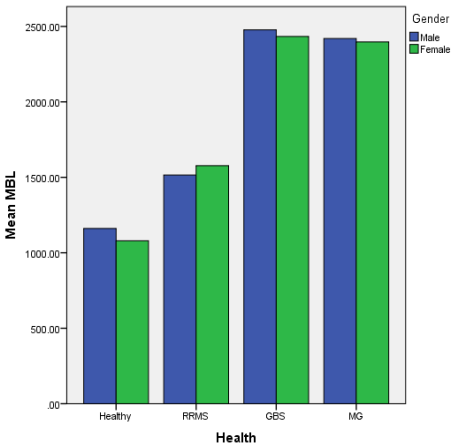

Figure 1. The bar graph of MBL concentrations in serum of patients with RRMS, GBS, MG, and healthy controls.
MBL: Mannose binding lectin, RRMS: Relapsing remitting multiple sclerosis, GBS: Guillain-Barr'e syndrome, MG: Myasthenia gravis.

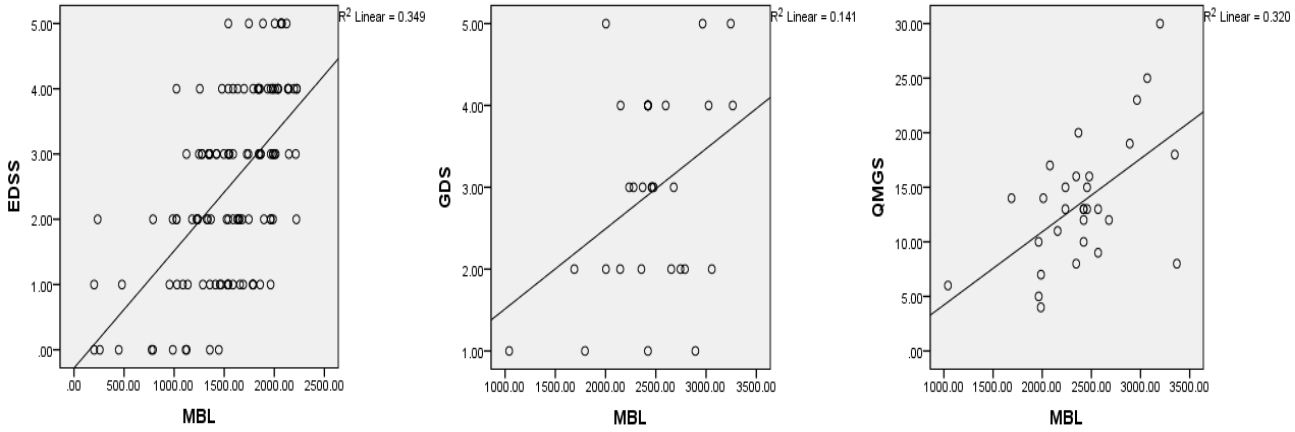

Figure 2. Correlation between serum level of MBL and severity MS, GBS, and MG.
MS: Multiple sclerosis, GBS: Guillain-Barr'e syndrome, MG: Myasthenia gravis, EDSS: Expanded disability status scale, GDS: GBS disability scale, QMGS: Quantitative myasthenia gravis score, MBL: Mannose-binding lectin.

However, we did not find a significant correlation between plasma levels of MBL and duration of disease and relapse number during last year (p=0.33 and p=0.42).

DISCUSSION

The complement system can be activated by MBL, a C-type lectin that belongs to the class of collections in the C-type lectin superfamily, whose function appears to be pattern recognition in the first line of defense in the pre-immune host.[23,24] The main aim of the present study was to investigate serum level of MBL in sera of patients with MS, GBS, and MG and also association between the mean serum concentration of MBL with severity of the diseases. To the best of our knowledge, limited studies have investigated the role of MBL complement pathway in the context of innate immune response in GBS, MG, and MS, especially by measuring MBL as a biomarker in CSF or serum of patients. Our results showed the contribution of MBL to the pathogenesis of MS which is in line with those earlier reported by Kwok et al.[8] They studied activation

of MBL pathway in 87 MS patients and non-MS controls by evaluation of the expression of MBL, MBL associated serine protease -2 (MASP-2), and functional MBL/MASP-2 mediated C4 cleavage (fMBL) in both plasma and cerebrospinal fluid (CSF) samples. They suggested that activation of MBL pathway has a role in the pathogenesis of MS as they found significant increase in the expression of fMBL and MASP-2 in plasma samples of MS patients compared to those of non-MS controls, but not significant differences regarding plasma levels of MBL and CSF levels of fMBL, MBL, and MASP-2. Therefore, their hypothesis as involvement of MBL pathway in the MS pathogenesis is based on the other important components of MBL pathway other than plasma or CSF levels of MBL, while our finding in regards to the activation of MBL pathway is mostly based on increased serum levels of MBL. However, it has been shown that complement activation in autoimmune demyelination has a dual role including the promotion of demyelination and protection of oligodendrocytes from apoptosis.[25] Furthermore, it has been shown that elevated activation of complement may have a pivotal role in the induction and extent of the post-infectious immune-mediated peripheral nerve damages in patients with GBS. In this regard, Geleijns et al.[26] investigated the correlation between serum level of MBL and MBL complex activity with the development and severity of GBS. Although they did not report significant differences between cases and controls in terms of serum levels of MBL, they concluded contribution of MBL to the extent of nerve damage in patients with GBS. We also found a positive association between serum levels of MBL and severity of GBS, which supports previous hypothesis that in autoimmune disorders with antecedent infection such as rheumatoid fever and GBS, cross-reactive antibodies play a crucial role in mechanisms of pathogenesis including binding of MBL to damaged nerve tissue, followed by complement activation, attraction of inflammatory cells to the damaged site, and exacerbation of tissue injury.[26] MG is a prototypic antibody mediated autoimmune disease, characterized by widespread neuromuscular junction (NMJ) damage following complement activation and the lytic degradation of the postsynaptic NMJ membrane.[27,28] Accumulating lines of evidence on activation of complement in MG patients comes from studies that mostly investigated components of complement pathway other than MBL. However, Li et al.[29] suggested than MBL pathway is not involved in MG pathogenesis, which is not consistent with our findings. They found that there are no significant differences between anti-AChR antibody positive generalized MG patients and healthy controls in terms of MBL serum levels. Furthermore, they showed that AChR-immunization in MBL gene deficient mice can lead to considerable anti-acetylcholine receptor (AChR)-immune response and NMJ damage. Importance of complement system in the pathogenesis of MG has been suggested by the very first investigators, who earlier reported alteration in the serum levels of complement component in MG patients. Furthermore, the localization of complement deposits on segment of postsynaptic membrane has been demonstrated by immunohistochemistry methods.[30] However, the exact role of MBL in the pathogenesis of MG and other autoimmune neurological disorders has not been investigated in several studies.

In conclusion, the plasma level of MBL was found to be significantly increased in patients with MS, MG, and GBS compared to healthy controls. Furthermore, plasma level of MBL was significantly correlated with severity of these autoimmune neurological disorders. Our findings suggest that complement activation mediated by MBL contributes to the pathogenesis and also severity of MS, MG, and GBS. However, because the lectin pathway can be involved in several phases of the immune response, further evidence will be required to elucidate the underlying mechanism.

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