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Full Length Research Paper

Elevated antibody titer against *Borrelia burgdorferi* in new case of multiple sclerosis patients

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Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease that affects central nervous system (CNS). The etiology of MS is unknown, but it likely involves a combination of genetic, non-genetic and environmental factors such as microbial agents. In this descriptive case- control study, 61 new cases of MS patients and 60 healthy controls were studied for IgM and IgG antibody titer to *Borrelia burgdorferi*. The samples were tested for the presence of specific antibodies by ELISA method. The data were analyzed using descriptive statistics. The results showed that specific IgM antibody titer against *B. burgdorferi* were significantly higher in patient group than control (OR=4.323, 95% CI=1-6.4, p=0.001). Our results confirm the association between *B. burgdorferi* and MS in Iranian patients.

Key words: Antibody, *Borrelia burgdorferi*, multiple sclerosis.

INTRODUCTION

Multiple sclerosis is an autoimmune disease characterized by the inflammatory demyelination of neurons in CNS (Lutton et al., 2004). It has been hypothesized that MS results when an environmental agent or event (for example, virus, bacteria, chemicals, lack of sun exposure) acts in concert with a genetic predisposition to immune dysfunction (Sospedra and Martin, 2005; Mechelli et al., 2010). MS primarily occurs in women with the onset of clinical symptoms occurring between 15 and 50 years of age. The incubation period of MS is 7.2 years and the development of disease is associated with migration to high- risk area after age 11 (Wingerchuk and Weinshenker, 2000). The molecular mimicry pattern of MS revealed that auto antibodies and auto reactive T cells against myelin antigens such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) have been detected in MS patients play an important role in the etiology of the MS (Prat and Martin, 2002). Data obtained from many researches consider MS as a CD4+T helper-1(Th1)-mediated inflammatory demyelinating disease (Sospedra and Martin, 2005; Lovett-Racke et al., 2011). However, over the years a large number of different viruses have been linked to MS (Meinl, 1999; Wingerchuk and Weinshenker, 2000; Khaki et al., 2011). In addition, to viral infection, bacterial infection with *B. burgdorferi*, the spirochaete responsible for Lyme disease can involve central nervous system and may mimic the clinical symptoms of MS (Karussis et al., 1999 Fritzsche 2005). There are conflicting results as regards the seroepidemiological studies of *B. burgdorferi* and MS. Chmielewska-Badora et al. (2000) found that ten of 26 (38%) patients with MS were seropositive for *B. burgdorferi* compared with 149 of 743 (20%) patients with other neurological disorders (Chmielewska-Badora et al.,

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Table 1. Demographic characteristics of MS patients and control group.

Groups	Number	Gender male/female	Mean age± SD*
MS patients	61	9/52	32 ± 17
Control group	60	10/50	35 ± 15

*SD; standard deviation.

Table 2. The seroprevalence of antibodies against *B. burgdorferi* in Iranian patients with multiple sclerosis.

Groups / Anti-bacterial antibodies	MS[1] Patient n=61 SP/SN (Mean ± SD)[4]	Controls n=60, SP/SN (Mean ± SD)	OR[2] (95% CI)	P. value (SP/SN)[3]
Anti- *B. burgdorferi* IgM≥ U/ml	24/61(19.9 ± 9.5)	8/60(7.01 ± 6.8)	4.32(1-6.4)	0.012
Anti- *B. burgdorferi* IgG≥U/ml	10/60(7.7 ± 3.4)	12/60(7.8 ± 5.8)	-	N.S

1; Multiple sclerosis, 2; Odds ratio, 3; Sero positive/Sero negative, 4; (Mean of antibody ±standard deviation).

2000). Nevertheless, others reported negative findings (Schmutzhard et al., 1988; Coyle, 1989).

On the other hand, it has been shown that lyme borreliosis can mimic central nervous system lymphoma (Batinac et al., 2007). It is related to the release of quinolonic acid which is a neuro-toxin that is produced in both Lyme patients and MS patients as a result of macrophage activation in the CNS. Quinolonic acid production and myelin destruction is a very specialized form of inflammation in the brain that does not involve lymphocytes as is part of the inflammation process (Guillemin et al., 2003; Hartai et al., 2005).

This study was carried out in the central and west-central provinces of Iran, where there is increasing frequency of MS; therefore evaluation of etiologic risk factors (bacterial systemic infections particularly *B. burgdorferi* infection) is necessary. In this study, new case MS patients were selected. This group was not under medication or other interventional procedures. They had intact immunological profile and therefore microbial infections (detectable by antibody) can be considered as effective local risk factors, in MS risk. Therefore the aim of present study was to analyzing the relationship between the confirmed cases of MS and occurrence of positive serologic reaction to *B. burgdorferi.*

MATERIALS AND METHODS

Ethics approval

This study was approved by the Arak University of Medical Sciences Ethics Committee (AUMSEC-86-22/4).

The study included 61 new cases of MS patients and 60 of healthy controls. Serum samples were examined for antibodies against *B. burgdorferi* by ELISA method. This study included patients with a diagnosis of MS by the McDonald criteria (McDonald et al., 2001). The inclusion criteria were: at least 1 relapse in the past two years, more than 3 lesions on spinal or brain-MRI or both, baseline-Expanded Disability Status Scale (EDSS) from 0 to 3.5, age from 15 to 60 years. These patients were not taking any medication with known effect on immune system. Normal individuals were selected as a control group from the same ethnic background and geographic area. These subjects were matched for age, sex and socioeconomic status. All sera were tested by specific ELISA kits (IBL, Hamburg, Germany) for detection and measuring of antibodies (IgG and IgM against *B. burgdorferi*).

Statistical analysis

Non-parametric tests were employed since the study variables were abnormally distributed. The Kruskal–Wallis test was used to test for significant differences between group medians in the variables studied. The logistic regression was used to investigate relations between the various study variables and to predict the probability that the incidence of MS will occur as a linear function of one (or more) of the independent variables and to estimate the risk (odd ratio) of the disease. A probability value of less than 0.05 ($p < 0.05$) was considered as indicative of statistical significance. On the other hand, the descriptive variables are reported here as mean ± standard deviation. (Mean is measurement of each variable in all of patients and compared with control group). The all statistical analysis was performed using SPSS 16.0 (Chicago, IL, USA).

RESULTS

Patients group included 61 patients (9 men and 52 women) with the age range of 16-57 years and mean age of 32 ± 17 years and control group included 60 patients with the age range of 17-53 years and mean age of 35 ± 15 years. High frequency of MS was seen in 21 to 30 years group (35.5%). There was no significant difference between two groups in age, sex and socioeconomic status (Table 1). The anti-*B. burgdorferi* IgM was positive in 39.34% of the MS patients and in 13.3% of the control group (p=0.012, Table. 2). The prevalence of anti-*B.*

burgdorferi IgM was significantly higher in the MS patients than in the control group (OR=4.323, 95% CI=1-6.4, p=0.001). The IgG antibody to *B. burgdorferi* was found in 10 out of the 61 MS patients and in 12 out of the controls. There was no significant difference in seroprevalence of anti- *B. burgdorferi* IgG between these two groups (Table 2).

DISCUSSION

An infectious etiology of MS has been suspected for more than a century, results from different areas of investigation pointing in this direction. Although, Kurtz (1986) determined that this spirochete may be one of the major causes of MS. The opposite view is presented by Schmutzhard (Schmutzhard et al., 1988) negated any association between LB and MS.

In this study of MS patients, presence of acute antibody against *B. burgdorferi* was found more than 4 fold in patient group comparing with control group. There are some accomplice (Chmielewska-Badora et al., 2000; Fritzsche, 2005; Blanc et al., 2010) and some adverse studies with this finding (Coyle, 1989). For instance, Oksi et al. (1996) reported that increasing titer of antibody against *B. burgdorferi* is in agreement with MS but in other study just IgG anti- *B. burgdorferi* was detected in MS patients (di Bella et al., 1993). The specific property of the present study is selection of new case of MS, because different phase of infection may evoke different immune response. In this study IgM anti-*B. burgdorferi* in MS group is significantly more than control group. In under considerate MS patients, history of acute Lyme infection more than 4 fold may caused by social and behavioral activities in low level health area.

One of the basic justifications for these differences is diversity of epidemiological and biological criteria (race, social economical situation, and rural-urban communications). Vector borne diseases such as Lyme infection is increased in these situations. As well as preliminary it is noticeable that the difference between our findings and other researches may depend on difference of HLA allotypes of our cases. Stereotyping of Lyme agent in under studied area may present competed data for bacteriological investigations.

The nature of the stated relationship between infection with LB agent and MS disease remains the issue to be debated. In some cases, there are possibilities that neuroborreliosis may be misdiagnosed as MS. A direct provoking of MS by *B. burgdorferi* does not look probable, but it cannot be excluded that the LB agent may aggravate the pathogenic processes in the initial stage of MS and thus increase a number of the symptomatic, clinically diagnosed cases. The hypothesis by Karussis et al. (1999) proposed similar steps contributing in pathogenicity of the both disease, supported by other studies: activation of the lymphocytic System (Heller et al., 1990), inducing of the matrix metalloproteinase (MMPs) production (Perides et al., 1999), inducing of the auto antibodies production, including antibodies to neuronal proteins (Kaiser, 1995) and to myelin basic protein.

Several studies suggest a correlation between Lyme infection and risk of MS (Batinac et al., 2007). In these studies cerebral lymphocytic vasculitis and multifocal encephalitis could be associated with *B. burgdorferi* infection. The presence of this agent in inflammatory tissue indicates that direct invasion of *B. burgdorferi* may be the pathogenetic mechanism for focal encephalitis in Lyme neuroborreliosis. According to these hypotheses, we suggest a thorough study of cerebrospinal fluid in the patients suspected to MS and Lyme disease (Oksi et al., 1996; Batinac et al., 2007).

Conclusion

According to stability of epidemiological condition and increasing frequency of MS in this study, it is necessary to recognize the life cycle of Lyme infection in similar epidemiological area specially vector colonization. Web of causation epidemiological model is one of the basic methods for recognition and controlling of infection. From the point of view of hygiene, community health education and promotion of health situation is the most important governmental intervention for controlling of these complexities.

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