



Investigation of *Chlamydia pneumoniae* Infection in Patients With Multiple Sclerosis: A Case-Control Study

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Abstract

Background: *Chlamydia pneumoniae* has currently been proposed to be involved in multiple sclerosis (MS) pathogenesis. The aim of this study was to evaluate the serum level of anti-*C. pneumoniae* immunoglobulin G (IgG) in patients with MS compared to the control group, which may be helpful in further understanding of MS etiology.

Methods: The serum was obtained from 66 people with MS and 20 healthy people as patient and control groups, respectively. Serums were matched to the patient's age, gender, and place of residence. Finally, anti-*C. pneumoniae* IgG was measured by a sandwich enzyme-linked immunosorbent assay (ELISA).

Results: Sixty-six patients (25 men and 41 women within the age range of 17-56 years and mean±SD of 35.53±9.56) with MS and 20 control subjects (8 men and 12 women with the age range of 25-56 and mean±SD of 38.3±9.66) were enrolled in this study. The mean serum anti-*C. pneumoniae* IgG of patients with MS (28.82±33.54 RU/mL) was compared with that of the control group (4.075±6.691 RU/mL) and a statistically significant difference was observed in this regard ($P<0.001$).

Conclusions: In general, there was a significant relationship between the incidence of MS and the *C. pneumoniae* infection. However, the actual involvement of *C. pneumoniae* in MS is still a matter of debate and needs to be clarified in further studies (e.g., by creating animal models and conducting trial tests).

Keywords: *Chlamydia pneumoniae*, Multiple sclerosis, ELISA



Background

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS) with myelin destructions induced by the immune system (1). The clinical symptoms of MS are highly variable and can include impaired vision and abnormalities in the motor and coordination systems (2). Four general forms for this disease include primary progressive, secondary progressive, relapsing-remitting, and progressive relapsing (3). The disease typically occurs at the age of 18 to 40 and almost in women twice as much as men (4). The incidence of MS in different regions of Iran varies from 5.3 to 74.28 per 100 000 people (5-8). Nevertheless, a study showed that the prevalence of MS was 61 patients per 100 000 people in Hamadan (9). Although the cause of this disease is still unknown, several factors such as genetics, environmental factors, and infectious agents (bacteria and viruses) are known in the etiology of this disease (10). Bacteria as one of the most important infectious agents

in human societies can be one of the factors contributing to MS (11). The potential of bacteria in molecular mimicry as an effective mechanism for inducing MS is more than that of viruses (12). Some studies evaluated the role of *Acinetobacter*, *Helicobacter pylori*, Spirochetes, *Campylobacter*, *Mycobacteria*, and *Chlamydia pneumoniae* in the etiology of MS (13-18). *C. pneumoniae* is a gram-negative, obligate intracellular, and non-motile bacterium which causes acute respiratory diseases like pneumonia (19). In addition, *C. pneumoniae* causes other diseases such as atherosclerosis, asthma, encephalitis, and arthritis (20). There are few studies on the association between MS and *C. pneumoniae* infection. Accordingly, the present study aimed to estimate the serum level of immunoglobulin G (IgG) anti-*C. pneumoniae* antibodies in MS patients compared with the control subjects. It is hoped that the findings of this study help further understand the etiology of MS.

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Methods

This study included 66 patients with MS who were admitted in the Neurology Department at Farshchian hospital of Hamadan province, Iran between December 2017 and August 2018. This disease was diagnosed by specialized neurologists based on McDonald's criteria (21) and 20 healthy people were matched with patients' age, gender, and place of residence.

Three milliliters of blood samples were collected from all individuals in the study and then were centrifuged at 3000 rpm for 7 minutes. The sera were stored at -70°C until further analysis. Then, *C. pneumoniae* IgG was measured by the ELISA kit (Euroimmun, Germany) according to the manufacturer's instructions.

Statistical Analysis

Data analysis was performed using SPSS software, version 16. *P* values less than 0.05 were considered statistically significant.

Results

In general, 66 patients (25 men and 41 women in the age range of 17-56 years and with the mean \pm SD of 35.53 \pm 9.56) with MS and 20 control subjects (8 men and 12 women within the age range of 25-56 and the mean \pm SD of 38.3 \pm 9.66) were enrolled in this study. Table 1 presents the anti-*C. pneumoniae* IgG and demographic characteristics in patients with MS and healthy subjects.

The mean serum anti-*C. pneumoniae* IgG of patients with MS (28.82 \pm 33.54 RU/mL) was compared with that of the control group (6.69 \pm 4.07 RU/mL) indicating a statistically significant difference (*P* < 0.001).

Discussion

MS is considered a chronic inflammatory disease that affects the CNS and causes several neurological disorders (22). Thus, this study investigated the relationship between MS and *C. pneumoniae* infection in a sample of the Iranian population. Although no evidence is available regarding the main cause of the disease, some epidemiologic studies confirmed the role of genetic factors and the environment

in the development of the disease (23-26). Therefore, the role of infectious agents as environmental factors is important from this perspective (11). *C. pneumoniae* is one of the most important organisms and its role has been recently emphasized in MS. Due to the inability of the host to complete the elimination of *C. pneumoniae*, this bacterium can lead to persistent infections (27).

Monocytes pass the bacterium from the blood-brain barrier and enter it into the CNS, which ultimately causes inflammation. Therefore, it can play a role in the development of MS (28). Several studies have confirmed the association between MS and *C. pneumoniae* infection. In this study, it was found that anti-*C. pneumoniae* IgG in MS patients was significantly higher compared to the control group. The number of patients and the control group in this study was 66 (25 men and 41 women) and 20 (8 men and 12 women), respectively. In addition, the mean age of the patient and control groups was 35.53 and 38.3, respectively. In contrast to our study, Ramroodi et al showed that there is no direct correlation between MS and *C. pneumoniae* infection. In this study, the number of patients and healthy people was 80 and their average age was 36.63 and 36.53, respectively. Further, the mean age was similar to that of our study (29). In another study, Khaliel and Abbas also found no significant relationship between *C. pneumoniae* infection and MS disease. The mean age of people with MS and healthy people was 34.5 and 34.7, respectively, and the number of patient and control groups was 60 (25 men and 35 women) and 30 (8 men and 22 women), respectively (30). Similarly, Aghaei et al demonstrated no association between MS and *C. pneumoniae* in Iranian people. One reason for the discrepancy between the findings of this study and those of our study could be the difference in geographical areas. The number of patients and the control group in that study was 85 (18 men and 67 women) and 50 (12 men and 38 women), respectively, and their mean age was reported to be 33.8 and 33.9. Some studies (30-31) were similar to our study in that the number of people in the control group was more than that in the patients group (31). Munger et al found that there was a positive association between *C. pneumoniae* infection

Table 1. Characteristics of MS Patients and Control Subjects

Factors		Case Group (n=66)	Control Group (n=20)	<i>P</i> Value
Age	(mean \pm SD)	35.53 \pm 9.56	38.3 \pm 9.66	
	Range (years)	17-56	25-56	
Gender	Men, No. (%)	25 (37.9)	(40) 8	
	Women, No. (%)	41 (62.1)	12 (60)	
IgG anti- <i>Chlamydia pneumoniae</i> (> 22 RU/mL)	(mean \pm SD)	28.82 \pm 33.54	4.075 \pm 6.691	< 0.001

Note. MS: multiple sclerosis; SD: standard deviation; IgG: immunoglobulin G.

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and the progression of MS (32), which is in line with the findings of our study. Likewise, Sriram et al showed that *C. pneumoniae* infection in MS patients was more than that of the control group (20). Another study concluded that *C. pneumoniae* infection was more frequent in MS patients compared to the healthy population (33). An experimental model of MS was done on rats and the results showed that the peptides of this bacterium are similar to myelin (34). Further, Layh-Schmitt et al reported that *C. pneumoniae* can be found in the cerebrospinal fluid of MS patients (35). Currently, *C. pneumoniae* has been proposed to be involved in the pathogenesis of MS, and thus more studies with higher sample sizes are needed to further demonstrate the role of *C. pneumoniae* in the development of MS. However, the actual involvement of *C. pneumoniae* in MS is still a matter of debate and needs further clarification in future studies (e.g., by the creation of animal models.)

Conflict of Interests

None.

Ethical Approval

This study was confirmed by the Ethics Committee of Hamadan University of Medical Sciences (Ethical approval code: IR.UMSHA.REC.1396.187).

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