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Chlamydia pneumoniae seropositivity in Iranian patients with multiple sclerosis: a pilot study

Obecność przeciwciał przeciw Chlamydia pneumoniae wśród irańskich chorych na stwardnienie rozsiane: badanie pilotażowe

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Abstract

Background and purpose: Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system. Genetic and environmental factors could not completely explain the pathogenesis of the disease. Among environmental factors, infectious agents are of more interest than other candidates, so *Chlamydia pneumoniae* (*C. pneumoniae*) may have a role in MS development or progression. This study aimed to evaluate *C. pneumoniae* seropositivity in MS patients.

Material and methods: Serum samples obtained from a cohort of 85 patients with MS and from 50 age- and sex-matched controls were assessed for the presence of antibodies. IgM and IgG concentration for *C. pneumoniae* were determined with enzyme-linked immunosorbent assay (ELISA).

Results: The mean age was 33.8 (9.96) years in the MS group and 33.9 (10.7) years in controls. Female/male ratio was 3.5 : 1 in the MS group; 69 patients (81%) had relapsing-remitting course (RRMS) and 16 patients (19%) had secondary progressive course (SPMS). The median concentration of *C. pneumoniae* IgM in the MS group was 0.5 RU/mL (0.25-1) versus 0.5 RU/mL (0.3-0.8) in the control group ($p = 0.66$); likewise, the median concentration of *C. pneumoniae* IgG in MS patients was 57.3 RU/mL (17.05-95.1) compared with 56.15 RU/mL (6.85-102.5) in the control group ($p = 0.85$).

Streszczenie

Wstęp i cel pracy: Stwardnienie rozsiane (SR) jest przewlekłą zapalną chorobą autoimmunologiczną ośrodkowego układu nerwowego. Czynniki genetyczne i środowiskowe nie tłumaczą w pełni patogenezy choroby. Wśród czynników środowiskowych szczególne zainteresowanie budzą drobnoustroje powodujące zakażenia. *Chlamydia pneumoniae* (*C. pneumoniae*) mogłaby odgrywać rolę w powstawaniu lub postępie SR. Celem badania była ocena obecności przeciwciał przeciwko *C. pneumoniae* w surowicy chorych na SR.

Materiał i metody: Od 85 chorych na SR i od 50 osób z grupy kontrolnej, dobranych pod względem wieku i płci, pobrano krew i za pomocą testów immunoenzymatycznych (ELISA) zbadano stężenia IgM i IgG przeciwko *C. pneumoniae* w surowicy.

Wyniki: Średnia wieku wyniosła 33,8 (9,96) roku w grupie chorych na SR i 33,9 (10,7) roku w grupie kontrolnej. W grupie chorych na SR proporcja kobiet do mężczyzn wyniosła 3,5 : 1. U 69 pacjentów (81%) choroba miała przebieg nawracająco-zwalniający, a u 16 pacjentów (19%) – wtórnie postępujący. Mediana stężenia IgM przeciwko *C. pneumoniae* wyniosła 0,5 RU/ml (0,25–1) w grupie chorych na SR w porównaniu z 0,5 RU/ml (0,3–0,8) w grupie kontrolnej ($p = 0,66$). Mediana stężenia IgG przeciwko

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Regarding the clinical course, *C. pneumoniae* IgG was 55.1 RU/mL (20.7-88.6) in RRMS and 59.1 RU/mL (5.35-112) in SPMS ($p = 0.8$).

Conclusion: No association was observed between MS and *C. pneumoniae* in Iranian MS patients.

Key words: multiple sclerosis, *Chlamydia pneumoniae*, ELISA.

C. pneumoniae wyniosła 57,3 RU/ml (17,05–95,1) w grupie chorych na SR w porównaniu z 56,15 RU/ml (6,85–102,5) w grupie kontrolnej ($p = 0,85$). Mediana stężenia IgG przeciwko *C. pneumoniae* wśród chorych na nawracająco-zwalniające SR wyniosła 55,1 RU/ml (20,7–88,6), a w grupie chorych z postacią wtórnie postępującą choroby – 59,1 RU/ml (5,35–112) ($p = 0,8$).

Wnioski: Wśród irańskich chorych na SR nie stwierdzono związku między SR a występowaniem przeciwciał przeciwko *C. pneumoniae*.

Słowa kluczowe: stwardnienie rozsiane, *Chlamydia pneumoniae*, ELISA.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system (CNS). The pathological hallmark of MS is demyelinating plaques accompanied by gliosis around the blood vessels [1].

The aetiology of MS is still unknown but it has been postulated that both genetic and environmental factors are involved in the pathogenesis of this debilitating disease. Among environmental factors, infectious agents are of more interest than other candidates [2]. Over the past years, many infectious agents such as EBV [3] and HSV-6 [4] have been suggested as potential causes of MS but inconsistent results have been obtained [5]. During recent years many studies have proposed a possible role for *Chlamydia pneumoniae* (*C. pneumoniae*) in MS development [6]. Sriram found increased CSF antibodies to *C. pneumoniae* in 86% of MS patients compared with controls [6].

C. pneumoniae is an intracellular pathogen and a member of the *Chlamydia* family. It is a common cause of human respiratory disease and is transmitted from human to human by the respiratory tract with an incubation period of several weeks [7].

C. pneumoniae induces a persistent infection due to the inability of the host to eradicate the pathogen, leading to a chronic infection. Several studies show that *C. pneumoniae* may alter the ability of microglial cells to enhance the cytokine production [8]. Therefore, *C. pneumoniae* could be considered as a potential factor in the pathogenesis of many chronic CNS diseases such as Alzheimer disease [9], atherosclerosis, stroke [10] and MS [11].

The present study aimed to evaluate the seropositivity of *C. pneumoniae* in Iranian MS patients compared to controls.

Material and methods

This study was conducted in Isfahan, the second largest province of Iran, located in the central part of Iran at the latitude 30-34°N and longitude 49-55°E, with a population of more than 4 million.

People living in Isfahan are ethnically Persian, belonging to Caucasian ethnicity. The total number of patients suffering from MS in Isfahan was 1391 with the prevalence of 35.5 per 100 000, according to a study in the year 2006 [12]. The prevalence of MS in this area is significantly greater than in many other Asian countries [13].

Serum samples obtained from a cohort of 85 patients with MS and from 50 age- and sex-matched controls were assessed for the presence of antibodies. The control group was selected from healthy people. In both groups, 7 mL of blood was taken and after centrifugation frozen at –70°C. Presence and concentration for *C. pneumoniae* IgM and IgG were determined by the enzyme-linked immunosorbent assay (ELISA), using *C. pneumoniae* IgG and IgM kit (Euroimmun, Germany).

Our patients were randomly chosen from registered patients of MS Clinics affiliated to the Isfahan University of Medical Sciences. Tenets of the current version of the Helsinki Declaration were followed; institutional ethical committee approval was granted, and the nature of the trial was explained to the patients. After a detailed discussion with the neurologist, each patient signed an informed consent form.

The demographic data were analysed by Student's *t*-test. *C. pneumoniae* antibody levels (including IgM and IgG) were compared in the two groups of MS patients and controls by the Mann-Whitney *U*-test.

Table 1. Characteristics of studied groups

	Patients with multiple sclerosis (n = 85)	Controls (n = 50)	p-value
Age, years; mean (SD)	33.8 (9.96)	33.9 (10.7)	
Sex			
Women, *n* (%)	67 (78.8%)	38 (76%)	
Men, *n* (%)	18 (21.2%)	12 (24%)	
Disease duration, years; mean (SD)	5.8 (4.66)		
EDSS score, mean	2.5		
C. pneumoniae IgM positive (> 1.1 RU/mL)	14 (16.5%)	7 (14%)	0.8
C. pneumoniae IgG positive (> 22 RU/mL)	61 (71.8%)	35 (70%)	0.82
C. pneumoniae IgM level, median (interquartile range)	0.5 (0.3-0.8)	0.5 (0.25-1)	0.66
C. pneumoniae IgG level, median (interquartile range)	56.15 (6.85-102.5)	57.3 (17.05-95.1)	0.85

SD – standard deviation; EDSS – Expanded Disability Status Scale

Results

Eighty-five MS patients (67 females and 18 males) and 50 controls (38 females and 12 males) were included ($P > 0.05$). Female/male ratio was 3.5 : 1 in MS patients. The mean age was 33.8 (standard deviation, SD 9.96) years in the MS group and 33.9 (SD 10.7) years in controls. Sixty-nine patients (81%) had relapsing-remitting course (RRMS) and 16 patients (19%) had secondary progressive course (SPMS).

The most common presenting symptoms were (in order of decreasing frequency): optic neuritis, sensory symptoms and motor signs.

In the MS group, the mean interval between the first and second attack was 1.74 (SD 1.96) years, mean disease duration was 5.8 (SD 4.66) years, mean number of attacks per year was 1.40 (SD 0.83), and mean score of the Expanded Disability Status Scale (EDSS) was 2.5 (SD 1.80). A positive family history of MS was found in 13 patients (15.2%).

The median concentration of *C. pneumoniae* IgM in the MS group was 0.5 RU/mL (SD 0.25-1) versus 0.5 RU/mL (SD 0.3-0.8) in the control group ($p = 0.66$). Likewise, the median concentration of *C. pneumoniae* IgG in MS patients was 57.3 RU/mL (SD 17.05-95.1) versus 56.15 RU/mL (6.85-102.5) in the control group ($p = 0.8$) (Table 1).

C. pneumoniae IgG was 55.1 RU/mL (SD 20.7-88.6) in RRMS and 59.1 RU/mL (SD 5.35-112) in SPMS ($p = 0.8$).

Taken together, we did not observe any correlation between either *C. pneumoniae* IgG or IgM and EDSS, number of attacks and disease duration.

Discussion

Our study did not reveal any difference for the antibody response to *C. pneumoniae* between MS patients and controls.

Multiple sclerosis is an autoimmune disease which is more common in women than men; in our patients the female-to-male ratio was 3.5 : 1, similar to many other studies [14,15] but higher than neighbouring countries such as Pakistan and Iraq [13-16].

According to the geographical distribution of MS, Isfahan province is expected to have a low-risk prevalence due to the latitude of the area. However, the results of an epidemiological study showed an unexpected incidence, with the prevalence of 35.5/100 000 (medium risk) [12]. Although insufficient epidemiological studies have been performed over the past years in this province, it seems that the prevalence of MS has been growing in recent years rapidly. Therefore, a thorough evaluation of the environmental factors, in particular infectious agents, is warranted.

Recent evidence supported the notion that infections associated with MS are ubiquitous. Thus, it is plausible that the long-term presence of *C. pneumoniae* in the infected tissue is able to bring about chronic inflammation in the target organ [17]. The *Chlamydia* family in-

cludes four species: *C. trachomatis*, *C. pneumoniae*, *C. psittaci*, and *C. pecorum*. *C. trachomatis* and *C. pneumoniae* are common in humans but with different routes of transmission and clinical presentation [7]. *C. pneumoniae* has been reported to be associated with neurological diseases such as ischaemic stroke in young adults [18], Alzheimer disease and MS.

One study showed that the neuronal cell line may be markedly sensitive to *C. pneumoniae* [19] and it may play an important role in the aetiology of MS. However, the results of other studies are inconsistent, neither confirming nor excluding a possible role for *C. pneumoniae* in MS development and progression [20].

Another study investigating the serum and cerebrospinal fluid of MS cases for *C. pneumoniae* showed the production of *C. pneumoniae* oligoclonal band IgG only in a minority of MS patients [21]. On the other hand, Sriram isolated *C. pneumoniae* from the CSF of 86% of MS patients compared with 11% of controls [6]. The present study did not detect any association between *C. pneumoniae* antibodies and MS patients, even when evaluated with respect to the diseases stage. In contrast to our result, Parratt *et al.* found *C. pneumoniae* infection more commonly in MS patients than in controls, particularly early in the course of the disease [22]. Although some investigations have shown a probable association of *C. pneumoniae* with MS, we could not confirm the existence of an association. Similarly, Munger *et al.* did not find any evidence of an association, replicating and verifying our results [20].

Conclusion

No association was observed between MS and *C. pneumoniae* IgG and IgM antibodies in Iranian MS patients.

Disclosure

Authors report no conflict of interest.

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