

Is there a relationship between Parkinson's disease and *Chlamydia pneumoniae*?

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Abstract

Objectives: The aim was to investigate a possible relationship between *Chlamydia pneumoniae* and Parkinson's disease (PD).

Study Design: Serum samples obtained from a cohort of 51 patients with PD and from 37 age- and sex-matched controls were assessed for the presence of antibodies. The control group was selected from healthy people. In both groups, 5 mL of blood was taken and after centrifugation frozen at -80°C . Presence and concentration for *C. pneumoniae* IgM and IgG were determined by the enzyme linked immunosorbent assay (ELISA) and immunofluorescence (IFA), using *C. pneumoniae* IgG and IgM kit (Euroimmun, Germany).

Results: *Chlamydia pneumoniae* IgG was positive in 50 (98%) patients in ELISA study. *C. pneumoniae* IgG was positive in 34 (92%) control subjects in ELISA study. *C. pneumoniae* IgG positivity in patients was slightly higher, but the difference did not reach statistical significance ($P = 0.17$). No statistically significant difference was found between the patient and the control groups in IFA study ($P \geq 0.5$). *C. pneumoniae* IgM results (both ELISA and IFA study) was negative in the both PD group and control group.

Conclusion: The present study indicated that the associations between PD and *C. pneumoniae* was suggestive.

Key words: *Chlamydia pneumoniae*, etiology, Parkinson's disease

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Introduction

Parkinson's disease (PD) is a progressive neurological disorder characterized by various motor and nonmotor features that can have an impact on function to variable degrees.^[1] PD consists mainly destruction of nigrostriatal dopaminergic neurons.^[1] Although different mechanisms are responsible for the destruction, disease-causing mechanisms of the etiologic factors are similar. There are autosomal dominant and recessive inheritance property and sporadic subtypes of PD.^[2]

Environmental toxins, infectious agents, trauma, aging and genetic predisposition are responsible for the cell death of sporadic PD.^[2,3] Involvement of nigrostriatal dopaminergic neurons are associated with the motor symptoms of

disease. Resting tremor, bradykinesia, rigidity and gait disorder (postural instability) are the most common symptoms.^[1,2]

In previous studies, it has been proposed that the risk of PD may increase with influenza A virus infection and has been reported that underlying mechanism may be the formation of Lewy bodies and death of nigral neurons.^[4]

Chlamydia pneumoniae, a Gram-negative obligatory intracellular microorganism, has been recognized as a common respiratory pathogen, named Taiwan acute respiratory strain and identified in 1989. The seroprevalence of infection has

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been reported between 25% and 45% of adults. *C. pneumoniae* causes pharyngitis, sinusitis, otitis media, tonsillitis, laryngitis, bronchitis, and is considered as an important and common pathogen of otolaryngeal diseases.^[5]

The relationship between *C. pneumoniae* and neurodegenerative diseases such as Alzheimer's disease (AD), multiple sclerosis has been investigated in numerous previous studies.^[6,7] *C. pneumoniae* may play a role in protein deposition and apoptosis in the central nervous system.^[6]

To the best of our knowledge, there is not any study evaluating the relationship between *C. pneumoniae* and PD in the current literature. Our aim was to investigate a possible relationship between *C. pneumoniae* and PD.

Materials and Methods

Subjects

Our patients were randomly chosen from registered patients of PD clinics affiliated to the Kirikkale University, Faculty of Medicine. 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 detailed information, each patient signed an informed consent form.

Diagnosis of PD was based on the United Kingdom Brain Bank's criteria as bradykinesia in association with rest tremor, rigidity, or postural instability.^[8]

For each patient, a detailed history of motor and nonmotor symptoms of PD was obtained, and the clinical condition was rated with the Unified Parkinson's Disease Rating Scale (UPDRS) (motor/total) and Hoehn and Yahr (H and Y) stage scores assessed in the "ON" state.

Patients were divided into two groups; tremor dominant (TD) and a nontremor dominant (NTD).

Exclusion criteria included: (a) Patients and controls who have other neurodegenerative diseases, (b) patients and controls who have psychiatric diseases, (c) patients and controls who have an active infection

Laboratory study

Serum samples obtained from a cohort of 51 patients with PD and from 37 age- and sex-matched controls were assessed for the presence of antibodies. The sample size our study was selected with regard to our patient population. Furthermore, previous similar studies were considered. As far as possible, age- and sex-matched volunteer family members, volunteer health workers, without history of neurodegenerative diseases or acute respiratory tract infection were taken as controls.

Transactional analysis was responsible for the samples' analysis. He was blinded to the group the subjects.

In both groups, 5 mL of blood was taken and after centrifugation frozen at -80°C . Presence and concentration for *C. pneumoniae* IgM and IgG were determined by the enzyme linked immunosorbent assay (ELISA) and immunofluorescence (IFA), using *C. pneumoniae* IgG and IgM kit (Euroimmun, Germany).

Enzyme linked immunosorbent assay study

Patient samples were performed a dilution as 1:101 by a sample buffer containing IgG and Rheumatoid factor absorbents. After 10 min incubation at room temperature, concurrently with undiluted calibrators and positive-negative controls, 100 μl from these dilutions were inoculated to antigen coated wells. The next step was 30 min incubation at room temperature. Subsequently automatic washing of 3 times with a wash buffer have been performed. As a next step 100 μl of enzyme peroxidase conjugated anti-human IgM has been added to the wells. After 30 min incubation at room temperature the washing step has been reperformed. Addition of 100 μl chromogenic substrate was followed by 15 min lasting dark incubation and subsequent addition of 100 μl stop solution. Finally the photometric measurement of 450 nm was performed. Calibrator points were calculated for both patients and controls and a value of ≥ 0.71 was accepted as positive.

Immunofluorescence study

The IFA test was performed with TITERPLANE Technique (Indirect Immunofluorescence: A Standardized Technique for the Determination of Autoantibodies and Antibodies against infectious agents [Euroimmun, Germany]). Patient and control samples were performed a dilution of 1/100 by phosphate buffered saline (PBS).

Procedure of the test

The ready to use slides, which had protective coat removed when slides reached room temperature. From the diluted serum samples an amount of 30 μl were inoculated over the test sites for each patient by avoiding air bubbles. The slides were covered by biochips and left to 30 min incubation at room temperature. By the end of incubation biochip slides were washed by PBS and exposed to a 5 min incubation inside a PBS-tween containing chamber. By providing lack of air bubbles, 25 μl of fluorescent labeled anti-human globulins were inoculated on each test and control sites. During this step, the back sides of slides have been wiped and dried contrast to providing of the test face to remain moisture. By keeping away sunlight a 30 min incubation was repeated. By the end of incubation, biochip slides were washed by PBS and exposed to 5 min incubation inside a PBS-tween containing chamber. Followingly back sides

of the slides again wiped and dried. The slides which are prepared according to producer instructions undergone examination on $\times 40$ power by a fluorescent microscope synchronously by two different examiners. Degree of fluorescent staining had been scaled semiquantitatively as $+ 1 \pm 4$ (negative 0, positive control $+ 4$). Filter of the fluorescent microscope was 488 nm while of the colored filter was 580 nm.

There were fluorescing elementary bodies in case of positive anti *C. pneumoniae* antibodies. In each slide, ten samples were able to be examined. The tests were performed in accordance with the instructions of the manufacturers (Euroimmun, Germany).

Statistical analysis

The results were analyzed using the computer software SPSS version 16.0. (SPSS Inc., Chicago, IL, USA) $P < 0.05$ was considered statistically significant in all tests. Continuous variables were presented in titer of mean and \pm standard deviation. Categorical variables were expressed as proportions. The Student's *t*-test was used to test the differences in continuous variables and Chi-square test was used for categorical values. Pearson correlation test was used to analyze the relationship between *C. pneumoniae* IgM and IgG and the duration of PD, UPDRS III score, H and Y stage score and the motor subtype.

Results

There were 51 PD patients (19 females and 32 males) and 37 controls (15 females and 22 males) in this study. The mean age was 67.5 ± 11.2 years in the PD group and 66.7 ± 10.5 years in controls. No statistically significant difference was found between the patient and the control group in terms of age and gender ($P > 0.05$) [Table 1]. There were 35 TD, 16 NTD patients in this study.

Enzyme linked immunosorbent assay study results

Chlamydia pneumoniae IgG was positive in 50 (98%) patients. *C. pneumoniae* IgG was positive in 34 (92%) control subjects. *C. pneumoniae* IgG positivity in patients was slightly higher, but the difference did not reach statistical significance ($P = 0.17$).

Chlamydia pneumoniae IgM results (both ELISA and IFA study) was negative in the both PD group and control group.

Immunofluorescence study results

Results were summarized in Table 2.

There was no relationship between *C. pneumoniae* IgM and IgG and the duration of PD, UPDRS III score, HandY stage score and the motor subtype (both ELISA and IFA study).

Table 1: Demographic characteristics

	Patients (n=51)	Controls (n=37)	P
Age, year	67.5 \pm 11.2	66.7 \pm 10.5	0.72
Gender (female, male)	19/32	15/22	0.75
PD duration, year	4.6 \pm 3.9		
UPDRS III score (while ON)	19.0 \pm 10.7		
H and Y score (while ON)	2.27 \pm 1.04		

PD=Parkinson's disease; UPDRS=Unified Parkinson's Disease Rating Scale; H and Y=Hoehn and Yahr

Table 2: IFA test results for *C. pneumoniae* IgG

	Patients (n=51) n/%	Controls (n=37) n/%	P
<i>C. pneumoniae</i> IgG+1	13/25.5	3/8	0.5
<i>C. pneumoniae</i> IgG+2	21/41	16/43	0.9
<i>C. pneumoniae</i> IgG+3	14/27.5	14/38	0.5
<i>C. pneumoniae</i> IgG+4	3/6	4/9	0.8

IFA=Immunofluorescence; *C. pneumoniae*=*Chlamydia pneumoniae*

Discussion

Genetic mutations may cause the death of dopaminergic and nondopaminergic cells by the abnormal processing by ubiquitin-proteasome and autophagy-lysosomal of incorrectly folded proteins, oxidative stress, mitochondrial dysfunction, inflammation, apoptosis and other pathogenic mechanisms in the brains of PD.^[1]

It has been shown that *C. pneumoniae* may have a role in protein deposition and apoptosis in the central nervous system.^[6] Thus, *C. pneumoniae* may lead to PD. In this study, we tested the possible contribution of *C. pneumoniae* in the development of PD.

Chlamydia pneumoniae has been detected in brain tissues of Alzheimer patients. Balin *et al.* have shown that brains of AD patients were found to be PCR-positive for *C. pneumoniae*, particularly in the cerebral regions, the most affected part by AD.^[6,9] Little *et al.* have found that intranasal inoculation of *C. pneumoniae* in mice induced AD-like hallmarks in brains.^[10] Moreover, Hammond *et al.* have demonstrated that *C. pneumoniae* antibodies were identified in AD brains, colocalizing with plaques and tangles in vulnerable brain regions.^[11] *C. pneumoniae* in archival tissue of Alzheimer patients have not been shown in other studies.^[12,13]

Chlamydia pneumoniae antibodies have been detected in multiple sclerosis patients. One study has shown that the neuronal cell line may be markedly sensitive to *C. pneumoniae* and it may play an important role in the etiology of multiple sclerosis.^[14] Another study investigating the serum and cerebrospinal fluid of multiple sclerosis cases for *C. pneumoniae* has shown the production of *C. pneumoniae* oligoclonal band IgG only in a minority of multiple sclerosis

patients.^[15] Sriram *et al.* have isolated *C. pneumoniae* from the cerebro spinal fluid of 86% of MS patients compared with 11% of controls.^[16] Contrary to these, Aghaei *et al.* have not observed any relationship between multiple sclerosis and *C. pneumoniae*.^[17]

However, these studies strongly mention the need of further studies for providing more clear evidences between *C. pneumoniae* and multiple sclerosis and ADs.

Our study could not provide adequate evidences about *C. pneumoniae*'s role in PD etiology. We investigated *C. pneumoniae* antibodies by immunofluorescent study and ELISA and found them to be high both in patients and controls. Examination of *C. pneumoniae* antibodies both by ELISA and IFA is a strong feature of this study. High levels of antibodies may be an indicator of high prevalence of *C. pneumoniae* in our region. In a study originated from our country, high seropositivities were found among healthy adults in a specific region.^[18] One limitation of this study is that our study group was relatively small. Another limitation of this study is that *C. pneumoniae* antibodies only investigate in the blood.

As a conclusion, the probable linkage between *C. pneumoniae* and PD should be furtherly studied by direct detection of the agent in addition to serologic tests. This may require postmortem studies. In our study we could not find a relationship between PD and *C. pneumoniae* seropositivity. However, further studies including postmortem detection methods are needed to enlighten this subject.

In conclusions, the present study indicated that the associations between PD and *C. pneumoniae* was suggestive.

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