The etiological role of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infections in systemic lupus erythematosus of Iraqi female patients

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Abstract

Recently, Systemic lupus erythematosus (SLE) was considered as one of the autoimmune diseases that the genetic and environmental factors contributed in the disease etiological profile. According to the environmental factors, infectious agents have been concluded to have a role in the etiology and pathogenesis of SLE. Chlamydia pneumoniae and Mycoplasma pneumoniae are among these infectious agents that have been suggested to be involved in the etiology of SLE. Accordingly, the current study was designed to assess the anti-C. pneumoniae and anti-M. pneumoniae IgG antibody status by enzyme linked immunosorbent assay (ELISA) in the sera of 64 Iraqi SLE females' patients and 32 Iraqi healthy females as controls.

The patients' group were distributed into two equal subgroups (32 cases in each group); arthritis and nephritis. The findings revealed that 25% of the total SLE patient's sera were positive for anti-C. pneumoniae IgG antibody, while such antibody was not detected in controls. The difference was significant ($p = 9.8 \times 10^{-4}$) and associated with OR of 22.1. With respect to M. pneumoniae, 67.7% of SLE patient's sera were positive for anti-M. pneumoniae IgG antibody while none of control's sera was positive. The difference was significant ($p = 4.0 \times 10^{-9}$) and the associated OR was 141.1. For both pathogens, a similar profile was observed in arthritis and nephritis SLE patients. These findings may suggest that C. pneumoniae and M. pneumoniae are two pathogens involved in etiology and pathogenesis of SLE.

Keywords: Anti-*Chlamydia pneumoniae* IgG antibody, Anti-*Mycoplasma pneumoniae* IgG antibody, Infectious agents, Systemic lupus erythematosus, Autoimmune disease.

Introduction

Systemic lupus erythematosus (SLE) is one of the autoimmune disorders in which genetic factors play a critical role in determining the disease incidence. However, an absence of tolerance for self-nuclear antigens is additionally considered to have a suitable role in the disease etiology[25,37]. It has been described that 40% of SLE patients are defective in clearing apoptotic cells, which are normally removed rapidly by phagocytes in healthy individuals[22]. A further etiological evidence suggests that environmental factors are required to trigger the disease in genetically predisposed individuals; especially females, in which sex hormones strongly influence the disease pathogenesis[23,26]. This may lead to an irreversible breakdown in immunological tolerance manifested by an immune response against endogenous nuclear antigens[34].

The concept of relationship between environmental factors and autoimmunity has significantly increased during the last years. Among the environmental factors that have been suggested to have a role in etiology of SLE are the presence of different infectious agents; for instance, viral infections (Epstein-Barr virus; EBV, retroviruses, herpes simplex virus I and II; HSV-I and II, paramyxovirus, cytomegalovirus, parvovirus B19 and coronavirus)[1,2,7,10].

In addition, bacterial infections (*Chlamydia trachomatis*, *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Escherichia coli*, *Streptococcus pneumoniae*, *S. pyogenes*, *Proteus mirabilis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) have also been suggested[1,3,4,12,15,24,36].

Also, a recent observation suggested that the protozoon parasite *Toxoplasma gondii* may be one of the etiological agents for developing SLE[1,14]. Molecular mimicry is one of the proposed theories that explains the role of these agents in triggering SLE. A bacterial cell wall or flagellar components sharing amino acid sequences with self-amino acid sequences of host may initiate cross-reactive T- and B-cell responses[17]. In this situation, autoreactive T-cells with T-cell receptors that recognize both a foreign peptide (bacterial peptide) and a self-peptide (host peptide) have been delineated[2,29].

In the present study two bacterial species were suspected to have role in etiology of SLE; they are *Chlamydia pneumoniae* and *Mycoplasma pneumonia*. *C. pneumoniae* is a gram-negative bacterium and an obligate intracellular widespread respiratory pathogen. It is a member of the family Chlamydiaceae that causes pharyngitis, bronchitis, sinusitis and pneumonia[31]. Previous studies referred that *C. pneumoniae* can be transmitted from person to person

without any reservoir[35]. It has also been reported that *C. pneumoniae* might be involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and SLE[19,30].

Mycoplasma pneumoniae is considered as the smallest self-replicating microorganism, characterized by lacking the cell wall. It causes upper and lower respiratory infections that may affect multiple organs in children and adults[5]. The first step of *M. pneumoniae* infection involves adhering to ciliated respiratory epithelium by P1 protein[18]. This reaction can damage the epithelial cells through secreting toxic substances such as hydrogen peroxidase and superoxide radicals. Then the local immune cells are induced to produce cytokines as inflammatory mediators of the infection[18,35]. The severity of the disease depends on the host immunological response and the extra-pulmonary complications may include some organs. As a consequence, autoimmune diseases might be initiated[32]. Serological examinations are the most common laboratory detection of *M. pneumoniae*[30,31] and recently molecular techniques have also been used to detect the microorganism[6,9,33].

Therefore, the present investigation aimed to investigate the role of *C. pneumoniae* and *M. pneumoniae* in etiology of SLE in a sample of Iraqi female patients. Such aim was achieved by determining anti-*C. pneumoniae* and anti-*M. pneumoniae* IgG antibodies in the sera of the enrolled patients. Two clinical manifestations of the disease were also considered in these assessments: nephritis and arthritis.

Materials and Methods

The study was approved by the ethics committee of Iraqi Ministry of Health, in which 64 SLE female patients were enrolled, with an age range of 23 - 36 years (32.5 ± 1.1 years). They were referred to Rheumatology unit at Baghdad Teaching Hospital during the period January – March 2016 for diagnosis and treatment. The diagnosis was made by the rheumatologists according to the 1997 revised criteria for SLE of the American College of Rheumatology (ACR). They are based on a clinical examination and a laboratory evaluation[38]. As suggested by the consultants, the patients were distributed into two clinical groups: nephritis and arthritis patients; each of 32 patients. In addition to patients, 32 apparently healthy women were also enrolled in the study (control group); matched patients for age (33.1 ± 1.4 years).

Sera of patients were first tested for anti-nuclear antibodies (ANA) and anti-double strand DNA antibody (dsDNA), which were detected by commercially available kits (Human Company, Germany). then, anti-*C. pneumoniae* and anti-*M. pneumoniae* IgG antibodies were detected by using commercially available kits (DEMEDITEC Company, Germany). The kits are based on the principles of ELISA. The microtiter strip wells (solid phase) are coated with either *C. pneumoniae* or *M. pneumoniae* antigens that allow the detection of anti-*C. pneumoniae* or anti-*M. pneumoniae* IgG antibody in sera of participants.

The data are given as numbers and percentage frequencies and significant differences between these frequencies were assessed by two-tailed Fisher's exact probability. Also, odds ratio (OR) and etiological fraction (EF) were also estimated. These statistical evaluations were carried out by using WIN PEPI software version 11.65.

Results and Discussion

All sera of SLE patients were positive for ANA and dsDNA antibodies, while none of the control sera were positive. For anti-*C. pneumoniae* IgG antibodies, it was found that 25% of SLE patients' sera were sero-positive while such antibody was not detected in controls. Such difference was significant ($p = 9.8 \times 10^{-4}$) and associated with OR of 22.1. In addition, the difference scored EF of 0.24 (table 1). Such profile was almost similar in both arthritis and nephritis SLE patients, but with a lower OR (19.1) among arthritis SLE patients and higher OR (26.3) among nephritis SLE patients and the differences were significant ($p = 0.01$ and 2×10^{-3} respectively) (table 1).

The presented findings suggest that anti- *C. pneumoniae* IgG antibody might be involved in the etiopathogenesis of arthritis and nephritis SLE patients, especially if we consider the OR values that had a range of 19.1 – 26.3. In statistical interpretation, individuals' positive for this antibody is at greater risk to develop SLE compared to individuals negative for this antibody; therefore *C. pneumoniae* infection might be considered as one of the infectious agents involved in etiology of SLE. Other research groups have focused on the role of *C. pneumoniae* infection in the etiopathogenesis of SLE and their results reported that *C. pneumoniae* might be involved in etiology and pathogenesis of SLE[16].

Also, it has been reported that the intensity of the disease was mostly presented in patients sero-positive for anti-*C. pneumoniae* IgG antibody than sero-negative SLE patients[11]. Their data also indicated that the frequency of positive anti-*C. pneumoniae* IgG antibody was significantly higher in SLE patients than that of controls[11,16]. However, further study was unable to confirm these findings and no association between *C. pneumoniae* infection and SLE development was suggested[8]. Such discrepancy leads further investigators to suggest further studies in this context to shed more light on the relationship between this microorganism and SLE development[21].

Testing anti- *M. pneumoniae* IgG antibody in sera of studied groups revealed that 67.7% of SLE patients were sero-positive for anti- *M. pneumoniae* IgG antibody; while such antibody was not detected in controls. Such difference was significant ($p = 4.0 \times 10^{-9}$) and the associated OR was 141.1 (table 2). A similar pattern of increased percentage was observed in nephritis and arthritis SLE patients, but the OR was different (121.5 and 160.8, respectively) (table 2).

These results strongly suggest that *M. pneumoniae* might be associated with etiopathogenesis of arthritis and nephritis SLE patients. The recorded OR values (range: 121.5 – 160.8) are in favor of such suggestion. The positive individuals for this antibody may be at a greater risk to develop SLE compared to negative individuals. Thus, *M. pneumoniae* infection might be supposed as one of the infectious agents that are associated with the etiology of SLE. Other studies have also focused on the role of *M. pneumoniae* infection in the etiopathogenesis of SLE and reached a similar conclusion[13,28].

Table 1
Anti-*C. pneumoniae* antibody status in total systemic lupus erythematosus patients and their extra-articular manifestation groups (arthritis and nephritis) and controls.

Anti- *C. pneumoniae* IgG antibody	Patients		Controls (N= 32)		OR	Etiological fraction	*p*	95% Confidence interval
	No.	%	No.	%				
Total SLE (No. = 64)	16	25.0	0	0.0	22.1	0.24	$9.8x10^{-4}$	1.3-365.7
Arthritis SLE (No. = 32)	7	21.9	0	0.0	19.1	0.22	0.01	1.1-335.5
Nephritis SLE (No. = 32)	9	28.1	0	0.0	26.3	0.28	$2x10^{-3}$	1.5-453.7

OR: Odds ratio; *p*: Two tailed fisher exact probability

Table 2
Anti-*M. pneumoniae* antibody status in total systemic lupus erythematosus patients and their extra-articular manifestation groups (arthritis and nephritis) and controls.

Anti- *M. pneumoniae* IgG antibody	Patients		Controls (N= 32)		OR	Etiological fraction	*P*	95% Confidence interval
	No.	%	No.	%				
Total SLE (No. = 64)	44	67.7	0	0.0	141.1	0.68	$4x10^{-9}$	8.6-2317.9
Arthritis SLE (No. = 32)	21	65.6	0	0.0	121.5	0.65	$6.3x10^{-9}$	7.1-2078.6
Nephritis SLE (No. = 32)	23	71.9	0	0.0	160.8	0.71	$3.8x10^{-10}$	9.3-2776.2

OR: Odds ratio; *p*: Two tailed fisher exact probability

A further earlier study also investigated the association of *M. pneumoniae* infection and SLE development and documented that not only anti-*M. pneumoniae* antibodies are associated with development of SLE, but the presence of anti-phospholipid antibody can have synergistic effect with *M. pneumoniae* infection to develop SLE[27].

Others investigators analyzed the relevance of human *M. pneumoniae* infection in SLE patients by defining the contribution of tumor necrosis factor (*TNF*), mannose-binding lectin (*MBL*) and Fcγ receptor IIa (*FCGR2A*) genes as clinical identifying genes and the study indicated that these genes and *M. pneumonia* are pre-disposing factors for SLE[20]. More recently, an increased titer of anti-*M. pneumoniae* IgG and IgM antibodies has been reported in sera of SLE patients who had no signs or symptoms and *M. pneumoniae* infection might have contributed to SLE development[39].

In conclusion, *C. pneumoniae* and *M. pneumoniae* are important pathogens that are associated with an increased risk to develop SLE in infected patients.

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