Detection of *Chlamydia pneumoniae* in Ankylosing Spondylitis Patients

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Abstract:

Ankylosing spondylitis is a complex debilitating disease because its pathogenesis is not clear. This study aims at detecting some pathogenesis factors that lead to induce the disease. *Chlamydia pneumoniae* is one of these pathogenesis factors which acts as a triggering factor for the disease. The study groups included forty Iraqi Ankylosing spondylitis patients and forty healthy persons as a control group. Immunological and molecular examinations were done to detect *Chlamydia. pneumoniae* in AS group. The immunological results were performed by Enzyme-Linked Immunosorbent Assay (ELISA) to detect anti-IgG and anti-IgM antibodies of *C. pneumoniae* revealed that five of forty AS patients' samples (12.5%) were positive for anti-IgG and IgM *C. pneumoniae* antibodies compared to controls which revealed seronegative. Molecular detection included *16srRNA* and *HSP-70* genes were to ensure the serological examination for detection of bacteria in the five blood samples which were positive; therefore, these results improved that *C. pneumoniae* played a role in the pathogenesis of the disease.

Keywords: *Chlamydia pneumoniae*, Ankylosing spondylitis, *16sRNA*, *HSP-70*.

Introduction:

Ankylosing spondylitis (AS) was defined as an inflammatory arthropathies relationship to HLA-B_{27} (1), and it is an immune-mediated inflammatory marker, autoantibodies formation was the hallmark of AS (2). There are many infectious agents that are related to AS such as mycobacterial, fungus arthritis (3, 4). In addition, recent studies refer to the association between autoimmune diseases and *C. pneumoniae* bacteria (5). AS disease caused inflammatory back pain which leads to structural and functional impairments (6). Because of the difficulties of *C. pneumoniae* isolation *in vitro*; therefore, ELISA and molecular techniques were used to detect the bacteria (7). Recent studies identify *C. pneumoniae* by DNA amplification of the unique region via using oligonucleotides primers specific for these regions (*16srRNA*, *OMP*, and *HSP-70* genes) (8, 9). While other studies reported the detection of anti-*Chlamydia pneumoniae* antibodies in the sera of AS patients (10, 11). The present study aims to detect the role of *C. pneumoniae* as a trigger factor for AS.

Materials and Methods:
Studied groups

This study includes forty Iraqi Arab AS patients (30 males and 10 females), who are

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diagnosed by the rheumatologist consultant of Baghdad Teaching Hospital in Baghdad city, and the matched numbers and gender of healthy persons as a control. The ethics committees of participating universities and the teaching hospital were approved in the study, and the information was obtained from all the studied groups.

Samples collection

Blood samples were collected from September 2016 to December 2016. Five milliliters of venous blood samples were collected from all studied groups, and the blood samples were divided into two parts, three milliliters put in silicon tube, to obtain the serum for the serological examinations, and the residual milliliters put in EDTA tube to isolate the DNA according to the manufacturer manual (Promega, USA) for molecular detection.

C. pneumoniae antibodies detection by ELISA technique

The immunological examination was done by using ELISA technique to detect the anti-*C. pneumoniae* IgG and IgM antibodies in the sera of studied groups according to the manufacturer leaflet (Human company, Germany).

DNA extraction

The whole DNA was extracted from the EDTA blood samples of the studied groups by using the manufacturer procedure of the commercial kit (Promega company, USA).

Molecular detection of *C. pneumoniae*

The molecular examination was done by using a polymerase chain reaction (PCR) technique to detect the virulence factors of *C. pneumoniae* (*16srRNA* and *HSP-70* genes) by using specific primers from Alpha DNA technologies company (Canada) (11) to amplify the target fragments of the chosen genes, PCR products were visualized by electrophoresing in 2% agarose gel (Conda company, USA) which was stained with red stain (Intron Biotechnology Inc., Korea). The size of appeared fragments was compared with a standard DNA ladder fragments size (KAPA™, Universal Ladder KK6302, USA), the primers sequences, PCR conditions, length of PCR products were listed in Table (1).

Table 1. the primers sequences, PCR conditions, length of PCR products (9)

Primers	Primers sequences	PCR conditions	PCR products size
16sRNA (F)	3-TGACAACTGTAGAAATACAGC-5	↓ Initial denaturation at 94ºC for 3 min. ↓ Denaturation (2) at 94ºC for 40 sec. ↓ Annealing at 60 ºC for 35 sec. ↓ Extension (1) at 72ºC for 35 sec. ↓ The steps from denaturation (2) to extension (1) were repeated 35 cycles. ↓ A final extension at 72ºC for 10 min.	238 bp
16sRNA (R)	3-ATTTATAGGAGAGAGGCG-5		
HSP-70 (F)	3-AAGTCGCTAAAGCTCCTACTG-5	↓ Initial denaturation at 94ºC for 5 min. ↓ Denaturation (2) at 94ºC for 40 sec. ↓ Annealing at 62 ºC for 35 sec. ↓ Extension (1) at 72ºC for 35 sec. ↓ The steps from denaturation (2) to extension (1) were repeated 35 cycles. ↓ A final extension at 72ºC for 10 min.	760 bp
HSP-70 (R)	3-CTTCATCAAAGTCGTCTCCA-5		

Results and Discussion:

The results of current study showed that the male: female ratio in AS group was 3:1 in which the age mean $\pm$ SE was 40 ± 3.0 years for this group that was compared to healthy control group with age mean $\pm$ SE matched to AS group (40 ± 4.3 years). Also, the recent findings appeared that five seropositive sera samples for anti-*C. pneumoniae* IgG and IgM antibodies (12.5%) of the forty sera samples of AS patients. There were significant differences ($P<0.05$) between these five seropositive samples (12.5%) and 100% seronegative of the healthy control group (Figure 1). The molecular diagnosis of *C. pneumoniae* in the blood samples of studied groups shows that these five seropositive of anti-*C. pneumoniae* IgG and IgM antibodies were positive for detecting the *16sRNA* and *HSP-70* genes by using specific primers, as shown in Figures 2 and 3, and Table (1). The 16sRNA fragment has a molecular size which was 238 bp, while the HSP-70 fragment has a molecular size 760 bp compared with a DNA ladder.
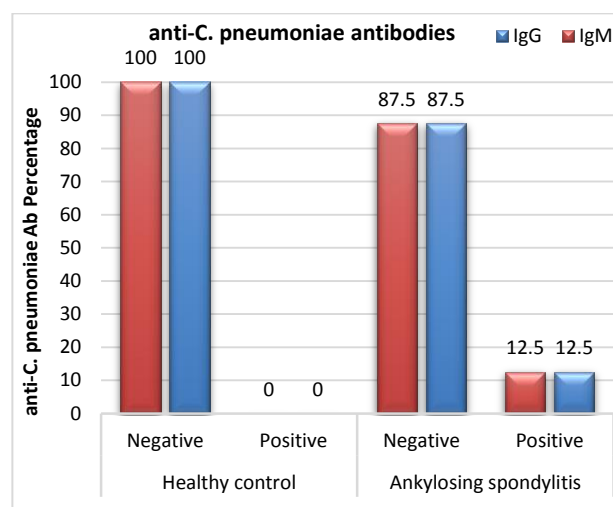
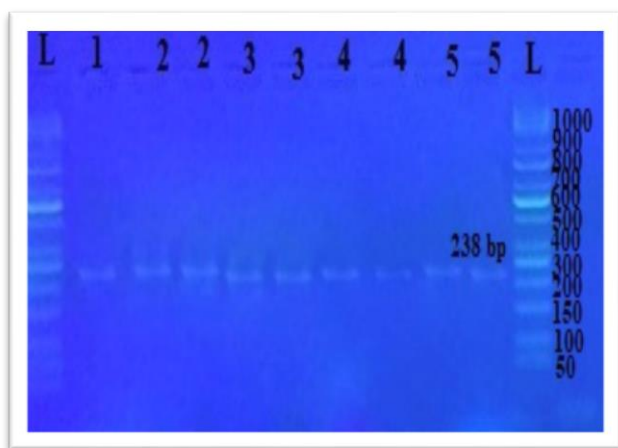**Figure 1. anti-*C. pneumoniae* antibodies in studied groups**

Figure 2. *16sRNA* **fragment gene in 2% agarose gel of the seropositive AS samples (L: DNA Ladder, 1, 2, 3, 4, 5: 16sRNA fragment of seropositive samples with duplicates)**
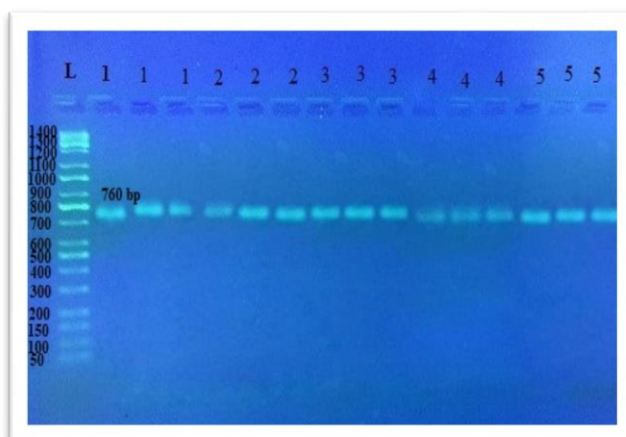

Figure 3. *HSP-70* **fragment gene in 2% agarose gel of the seropositive AS samples (L: DNA Ladder, 1, 2, 3, 4, 5: HSP-70 fragment of seropositive samples with duplicates)**

The detection of *C. pneumoniae* depends on the sensitivity of the diagnostic test because the cell wall structure of bacterium is more complicated. This leads to isolation and diagnosis difficulties (12); therefore, recent studies resort to use the molecular detection besides the immunological detection to ensure the causative and inducer agents of the disease (8, 13). The use of *16sRNA* gene fragment was to separate between the eukaryotes DNA and prokaryotes DNA, because all the prokaryotes have a *16sRNA* gene, while the eukaryotes have a *18sRNA* gene (8, 14). The analysis of DNA bands results appeared that five samples of the 40 AS group (12.5%) were positive to 16sRNA with molecular size 238 bp compared with the DNA ladder in an agarose gel as shown in Figure (1). This refers to the fact that these samples have the bacteria in the blood of patients. Such, HSP-70 can act as adhesive protein and play a major role in the pathogenesis of *C. pneumoniae*. It

has an association with ribosomal RNA subunit; because the later assumed the stabilization of their structure and HSP-70 has a role in antigen processing and presenting (11). The analysis of DNA band result of HSP-70 revealed that the same seropositive samples of AS group had a positive result to *HSP-70* gene fragment when it electrophoresed in agarose gel with molecular size 760 bp compared with the DNA ladder as shown in Figure (2). This result proved that these 5 samples have *C. pneumoniae* bacteria; because the *HSP-70* gene is a specific virulence gene for *C. pneumoniae* (15). Therefore, these results proved that *C. pneumoniae* had a role as a triggering factor in the pathogenesis of AS disease by induction mechanism.

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التحري عن *Chlamydia pneumoniae* في مرضى التهاب الفقار اللاصق

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الخلاصة:

يعد مرض التهاب الفقار اللاصق من الأمراض المنهكة المعقدة بسبب الامراضية الغير واضحة، صممت هذه الدراسة للكشف عن بعض العوامل المرضية التي تؤدي الى تحفيز المرض. أحد هذه العوامل المرضية هي بكتيريا *C. pneumoniae* التي تعتبر كمحفز لحدوث المرض. تضمنت الدراسة الحالية أربعين مريضاً عراقياً مصاباً بمرض الفقار اللاصق وعدد مماثل لأشخاص اصحاء اعتبروا كسيطرة. أجريت الفحوص المصلية والجزيئية للكشف عن الالتهاب الرئوي في عينات دم مجموعة الدراسة باستخدام تقنية طريقة الأنزيم المرتبط المناعية للكشف عن أضداد *C. pneumoniae* بنمطيها IgG وIgM. أظهرت النتائج أن 5 من 40 عينة مريض مصاب بمرض إلتهاب الفقار اللاصق (12.5%) كانت موجبة لكلا نمطي الأضداد مقارنة بمجموعة السيطرة السالبة لفحص الأضداد. أجري اختبار وجود جينات 16sRNA وHSP-70 والخاصة ببكتيريا ا*C. pneumoniae* للتأكد من وجودها في عينات دم المرضى الموجبة للفحص المصلي، والتي كانت إيجابية للاختبار الجزيئي. ومما تقدم، تؤكد النتائج الحالية أن لبكتيريا *C. pneumoniae* دور في إمراضيه المرض.

الكلمات مفتاحية: *Chlamydia pneumoniae*، مرض الفقار اللاصق، جين *16sRNA*، جين *HSP-70*.

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