Relationship between *Mycoplasma pneumoniae* infections and common autoimmune diseases

Hayder N. Ayyez , Habeeb S. Naher, Mohammed A. K.ALSAADI*

Department of Microbiology, College of Medicine, Babylon University Iraq,

*corresponding author:email:mbmc.kadhum70@gmail.com

الخلاصة:

ترافق الاصابة ب *M. pneumoniae* ظهور عديد من امراض المناعة الذاتية وانتاج الاضداد الذاتية و ان ٢٥% من الاشخاص المصابين بهذه البكتريا تحدث لهم مضاعفات في اعضاء خار ج الجهاز التنفسي وان المناعة الذاتية تلعب دورا في امراضيتها. تلعب خلايا Th17 دور في المناعة ضد *M. pneumoniae* وهذة الخلايا المناعية مسؤولة عن حدوث العديد من تفاعلات المناعة الذاتية وذلك لان الوظائف البيولوجية لل IL-17 تتصف بالطبيعة الالتهابية المزمنة و المدمرة.

اجريت الدراسة للكشف عن دور *M. pneumoniae* كعامل مساعد في حدوث المناعة الذاتية عن طريق قياس الاجسام المضادة من نوع IgG لتلك الجرثومة في مصل الاشخاص الذين لديهم واحد او اكثر من الاضداد الذاتية التالية ACPA ، ADSA ، ANA ،RF. كذلك قياس مستوى IL-17 في مصل اشخاص مصابين ب *M. pneumoniae* .

شملت الدراسة ثلاث مجاميع من الاشخاص المجموعة الاولى (أ) شملت ١٥٦ عينة مصل من اشخاص لديهم امراض مناعة ذاتية و الذين كانوا يراجعون قسم المناعة السريرية في مستشفى الصدر التعليمي في مدينة النجف حيث تم الكشف عن الاصابة عن طريق التحري عن اضداد *M. pneumoniae* من نوع IgG باختبار الايزا غير المباشر في الفترة من ايار الى تشرين الثاني ٢٠١٣ . اما المجموعة الثانية (ب) تألفت من ٤٠ عينة مصل من اشخاص مصابين ب *M. pneumoniae* بالاعتماد على قياس اضداد من نوع IgM . بينما شملت المجموعة الثالثة (ج) ٢٠ عينة مصل من اشخاص اصحاء ظاهريا للمقارنة.

كانت اعلى نسبة للاصابة ب *M. pneumoniae* بالاعتماد على قياس IgG في الاشخاص الذين لديهم اضداد ذاتية من نوع ACPA حيث كانت ٥٥,٥% وكانت نسبة الاصابة٥٦% في الاشخاص الذين لديهم اضداد ذاتية من نوع ADSA مقارنة مع الاصحاء والذين كانت نسبة الاصابة لديهم ٢٨,٥٧% بينما كانت نسبة الاصابة ٢٩,٤١% و ١٥,٧٨% في الاشخاص الذين لديهم الاضداد الذاتية من نوع ANA و RF على التوالي. من جانب اخر كانت هناك زيادة معنوية في مستوى IL-17 في الاشخاص المصابين ب *M.pneumoniae* والذين ظهرة عليهم الاعراض السريرية مقارنة مع الاشخاص المصابين بدون اعراض ظاهرية و الاشخاص الاصحاء.

وعلية قد تكون الاصابة ب *M. pneumoniae* عامل مساعد في تكوين الاضداد الذاتية من نوع ACPA و ADSA بعد الاستجابة المناعية لهذة البكتريا. كذلك فان الاصابة السريرية ب *M. pneumoniae* تؤدي الى زيادة معنوية ($P< 0.05$) في مستوى IL-17 .

Summary:

Background : *Mycoplasma pneumoniae* infection associated with the emergence of various autoimmune disorders and autoantibody production. Twenty five percent of patients infected with *M. pneumoniae* develop extra pulmonary complication and the autoimmune reaction thought to play a role in their pathogenesis. Many autoimmune diseases are believed to be Th17-mediated diseases, because the biologic functions of IL-17 are consistent with the chronic and destructive nature of inflammation.

Objective : This study was conducted to detect the role of *Mycoplasma pneumoniae* as cofactor in autoimmune reaction via measurement anti *Mycoplasma pneumoniae* IgG in the serum of patients that have one or more of the following autoantibodies anti-citrullinated protein antibody (ACPA), anti-double strand antibody (ADS), anti-nuclear antibody (ANA), and Rheumatoid factor (RF). Also measurement the level of IL-17 in the serum of infected patients with *M. pneumoniae*.

Material and methods : The study involved three groups of subjects, the first was 156 serum specimens (group A) were collected from patients suffering from autoimmune disorder admitted to clinical immunology department in Al Sadar Hospital in Al Najaf city, used in detection of anti-*M. pneumoniae* IgG by an indirect enzyme linked immunsorbent assay (ELISA) in the period from May to October 2013. While the second group (B) consist of 40 serum specimens from patients infected with *M. pneumoniae* (depend on anti *M. pneumoniae* IgM) and the third group was 20 apparently healthy control used for measurement level of IL-17.

Results : The prevalence of anti-*M. pneumoniae* IgG was at high percent in the patients have ACPA(55.5%) and ADSA (56%) compared with healthy control value (28.57%) while other patients have ANA (29.41%) and rheumatoid factor (15.78%) . There are significant increase in the level of IL-17 in the symptomatic infected patients with *M. pneumoniae* compared with asymptomatic infections and healthy control.

Conclusion : The *M. pneumoniae* infection may be cofactor in the production of ADS and ACP autoantibodies after infection and immune stimulation against this bacterium. Also there is significant ($P<0.05$) increase in the level of IL-17 in the symptomatic infected patients with *M. pneumoniae*.

Introduction :

Mycoplasma pneunoniae is a bacterium that infect the upper and lower respiratory tracts, cough, fever and headache may persist for several weeks[1]. Mycoplasmal cells have spherical to filamentous shape with no cell wall, there is an attachment organelle at the end of filamentous *M. pneumoniae* [1,2]. *M. pneumoniae* abilities to act as

polyclonal activator of lymphocytes and antibodies to various tissue and immune complexes are well known [3,4,5]. Although the exact mechanism of the extra pulmonary complications seen during the course of mycoplasma infections is not well understood, immune complex and autoantibody production are blamed for this entity[6]. Autoantibody production is probably due to the antigenic mimicry between the host and the microorganism or antigenic variation of the host cells occurring during the infectious process[7]. Antinuclar antibody (ANA) is one of the autoantibodies which is occasionally detected in sera of patients with *Mycoplasmal pneumoniae* [2,3].

Cold agglutinins was often associated with *M. pneumoniae* infection, and these autoantibodies were later characterized as recognizing *Ii* antigen of the human red cells, a carbohydrate antigen of surface glycolipids and proteins [8,9] . Host responses that develop after *M. pneumoniae* infection likely contribute to autoimmunity and variety of extra pulmonary complications involving the skin and nervous, cardiovascular ,renal, gastrointestinal, musculoskeletal and hematologic systems which occur in as many as 25% of infected persons[10]. The present study was conducted to reveal the role of *M. pneumoniae* infection in production of following autoantibodies ACP, ADS, ANA and RF. In addition to measurement level of IL-17 in serum of infected patients with *M. pneumoniae*.

Material and methods

The study was conducted in Al Najaf province which included 156 sera of patients aged from 18-55 year, all suffering from autoimmune diseases deepened on presence of the following autoantibodies ADS, ANA, ACCP and RF according to results provide from clinical immunology department in teaching Al Sadar hospital.

Three ml blood samples were collected from each patients, serum specimens were stored frozen. Serum samples were serologically investigated for *M. pneumoniae* specific IgG antibodies, using a commercial ELISA kit (Creative diagnostic USA) according to the manufacture instructions. On the other hand this study included measurement the level of IL-17 in three groups include symptomatic infections, asymptomatic infections (depend on presence anti-*M. pneumoniae* IgM) and apparently healthy control. Measurement of IL-17 was done according to the instructions of DuoSet ELISA kit / Sigma USA as following:

Plate preparation:

100µl of diluted Capture Antibody (4 µg/ml)) was coated into 96 –well micro plate. The coated plate was incubated at room temperature overnight.

The plate was aspirated and washed with Wash Buffer in washing machine special for ELISA for three times, Complete removals of liquid at each step were essential for good performance. After the last wash, the plates were inverted and blotted against clean paper towels.

Remaining protein binding sites were blocked by adding 200 µL of reagent diluents to each well (1%BSA in PBS). The plate was incubated at room temperature for minimum of 1 hour. After 1 hour the plate was washed 2 times with PBS.

Assay Procedure

A seven point standard curve has been made through 2-fold serial dilutions in reagent diluents and a higher standard of recombinant Interleukins was used at 2000 pg/ml.

One hundred µl of each standard (Interleukins) and serum were added into each well of 96 well plate and covered with an adhesive strip and incubated for 2 hours at room temperature.

After aspiration and washing like in step 2 of Plate Preparation, 100µl of the detection Antibody was added to each well, covered with a new adhesive strip and incubated for 2 hours at room temperature.

Aspiration/wash was repeated as in step 2 of Plate Preparation.

One hundred µl of the working dilution of Streptavidin-HRP was added to each well. The plate was covered and incubated for 20 minutes at room temperature.

After aspiration and washing like in step 2 of Plate Preparation, 100µl of substrate buffer solution was added into each well and incubated for 20 minutes at room temperature. Avoiding placing the plate in direct light. Fifty µL of Stop Solution was added to each well. The plate was gently tapped to ensure thorough mixing.

The optical density of each well was determined immediately using a micro plate reader set to 492 nm.

Statistical Analysis : The data analyzed using one way ANOVA test with least significant different under $P< 0.05$. Differences in the levels of IL-17 between patients and control were assessed by non-parametric statistical comparisons by ava computer system.

RESULTS:

The prevalence of anti *M. pneumoniae* IgG antibodies in patients have autoimmune diseases shown in table (1).

The results refer to relationship between *M.pneumoniae* infection , ADS and ACP autoantibodies. Also the result reflect to highest infection rate among patients have ADS autoantibodies also most positive cases were with high titer (200 to 300) . This refer to the relationship between *M. pneumoniae* infection and formation of this autoantibodies. The patients with ANA and RF autoantibodies reveal low infection rate 29% and 15.78% respectively and low effect on its production.

Table (1) : The prevalence of anti- *M. pneumoniae* IgG in the patients have following autoantibodies ADS, ACP, ANA, and RF.

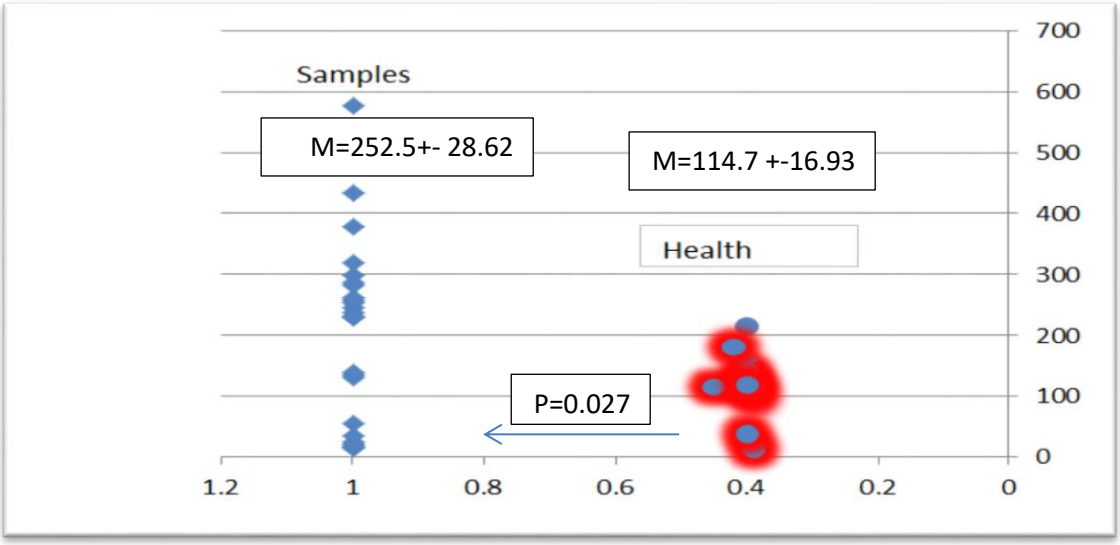
Type of autoantibody	Number of examined cases	Positive cases	Percentage
ADS [1]	50	28	56[a]
ACP [2]	36	20	55.55[a]
ANA [3]	34	10	29.41[b]
RF [4]	38	6	15.78[c]
Total	79	32	

The different between any two factor refers to the significant differences at $p < 0.05$.

[1] anti-double strand antibody [2] anti-citrullinated protein antibody

[3] anti-nuclear antibody [4] Rheumatoid factor

The results of this study showed the concentration of IL-17 in symptomatic infection group were significant increased as compared to control (252.5 +- 28.62 pg/ml , 114.7 +-16.93 pg/ml respectively), while in the asymptomatic infected patients there is non-significant increase as compared to control group (438.13 +- 188.94 pg/ml , 114.7 pg/ml respectively). This confirmed the role of this cytokines in this infection and severity of disease (figure 1 & 2).



Figure(1): Serum concentrations of IL-17 in *M. pneumoniae* infected patients of group A (median 252.5, 15-435) and Control (median 114.7, 12.6-214) P = 0.027 .

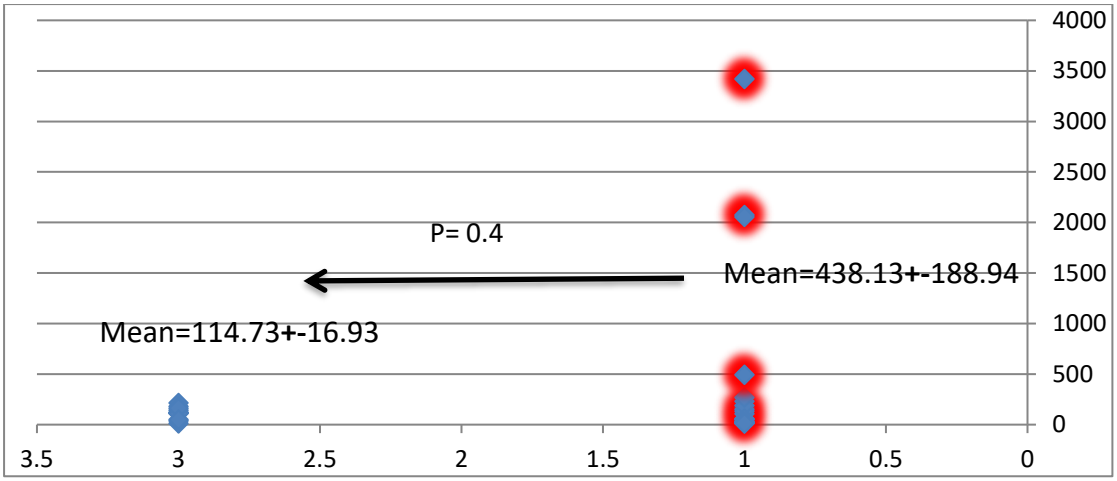


Figure (2): Serum concentrations of IL-17 in group B (median 438.13, 23-3418) and Control (median 114.7, 12.6-214) P = 0.464 .

Discussion:

Autoimmunity may arise by different mechanisms many of which as yet unidentified, one of these mechanism is molecular mimicry in which infection by particular microbes is associated with the subsequent development of specific autoimmune diseases [11].Cytadherence is consider to be the initial step in the virulent process of pathogenic mycoplasma. *M. pneumoniae* has adhisins that have an extensive sequence homology to mammalian structures. This molecular mimicry could generate autoreactive [12].

In this study we found significant differences at $P < 0.05$ in the prevalence of the serum antibodies (IgG) to *M. pneumoniae* among patients have ADS and ACP autoantibodies compared with patients having another autoantibodies including ANA and RF. Indicating that patients who have had infection with these microorganism have more risk to produce ADS and ACP autoantibodies. This agreement with Ramirez *et al.* when he revealed an association between the variable antibodies against *M. pneumoniae* and rheumatoid arthritis(RA) indicating that people who have had contact with this bacterium are about twice as likely suffering from Rheumatoid arthritis in which ACPA have proved to be powerful biomarkers that allow the diagnosis of rheumatoid arthritis (RA) to be made at a very early stage[13,14].

The pathogenic mechanism by which the diverse extrapulmonary symptoms subsequent to mycoplasma infection occur is thought to be possibly due to indirect tissue injury caused by an overzealous host immune response[15]. In this study we investigated the Th17 based immune response to mycoplasmal diseases using IL-17A as index markers. It was therefore suggested that extrapulmonary complications subsequent to the development of mycoplasmal pneumonia were due to breakdown of the immune response. This fact illustrates the results of this study in which the

concentration of IL-17 in symptomatic infection group significantly increased as compared to the control group, while in the asymptomatic infection group there is non-significant increase.

Kurata *et al*. confirmed that *M. pneumoniae* antigens promoted the production of IL-17A. Furthermore, in the presence of IL-6 and TGF-β1, IL-17A product by lymphocytes markedly increased in an antigen concentration dependent manner . IL-17A production by lymphocytes are induced by either *S. pneumoniae, K. pneumoniae* antigens or LPS which increased only twice as much as control in the presence of IL-6 and TGF-β1[15] . The addition of 50 μg protein/ml of *S. pneumoniae* antigens and 50 μg/ml LPS could not induce the levels of IL-17A as compared to *M. pneumoniae* antigens. Likewise, in mice infected with *Mycoplasma pneumoniae*, infiltration of the lung by neutrophils is dependent upon IL-23-induced upregulation of IL-17A and IL-17F ,the role of IL-17 in the host resistance against *M. pneumoniae* infection when he reported that *M. pneumoniae* of mouse lungs can be prolonged when IL-23 mediated IL-17 production is neutralized [17].

The differences between symptomatic infected and asymptomatic infected groups in the level of IL-17 may be depend on the bacterial dose, in acute and asymptomatic infection, which induce immune response as reported by Kurata *et al*. when he find severe inflammation was observed in the higher-dose and frequent sensitization group[17]. Intrapulmonary concentrations of IL-17A in mice were increased with higher amounts of *M. pneumoniae* antigens. The immunological response causes migration and generation of neutrophils which plays a part not only in host defence from bacterial infection but also as a pathological mechanism for autoimmune diseases such as chronic rheumatoid arthritis [17].

In the asymptomatic infected group of current study there is slightly elevation in level of this cytokine (non-significant) compared to the healthy individuals maybe also due to small bacterial colonization load leading to infer that *M. pneumoniae* enhancement of the Th17 response and the level of this cytokine may be determine disease severity and inflammation. There were Multiple studies report increased numbers of neutrophils in the lungs and bronchoalveolar lavage during *M. pneumoniae* infection suggest that the production of IL-17 during mycoplasma infections may induce the mobilization and recruitment of neutrophils[18].

References:

1-Cimolai, N.(1998). *Mycoplasma pneumonia*e respiratory infection. Pediatrics in Review, 19:327-332.

2- Srifuengfung, S.; Techachaiwiwat, W. and D hiraputra, C.(2004). Serological study of *Mycoplasma pneumonia*e infections. J Med Assoc Thia. 87(8): 935- 938.

3- Biberfeld, G. and Norberg, R.(1974). Circulating immune complex in mycoplasma infection. J. Immunol, 112:413-415.

4- Ruuth, E. and Praz, F.(1989). Interaction between mycoplasmas and the immune system. *Immunol*. Rev. 112: 133-160.

5- Stelmach, I.; podsiadlowicz-Borrzecka, M.; Grzelewski, T.; Magak, P.; Stelmach, W.; Jerzynska, J.; Boplawska, M. and Dziadk, J.(2005). Humeral in cellular immunity in children with mycoplasma pneumoinae infection: a 1-Year prospective study. Clinical and Dignostic laboratory immunology, 12(10): 1246-1250.

6- Hammerschlag MR. *Mycoplasma pneumonia*e infection. Lippincott Williams and Wilkins, inc. 2001; 14 (2):181-6.

7- Hammerschlag, M. R. (2001). Review *Mycoplasma pneumoniae* infections. Curr Opin Infect Dis, 14(2):181-6.

8- Yu LC, Twu YC, Chang CY & Lin M (2001) Molecular basis of the adult i phenotype and the gene responsible for the expression of the human blood group I antigen. Blood 98: 3840–3845.

9- Feizi T & Taylor-Robinson D (1967) Cold agglutinin anti-I and Mycoplasma pneumoniae. Immunology 13: 405–409.

10- Waites KB & Talkington DF (2004) Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev 17: 697–728, table of contents.

11- Doan T.; Melvold R.; Viselli S. and Waltenbaugh C. (2013). Immunology .Autoimmunity (16) 243-60.

12- Baseman JB., Tully JG. (1997). Mycoplasms: sophisticated, re-emerging and burdened by their notoriety. Emerg Infect Dis. 3: 21-32.

13- Ramireze, A. S.; Rossas, A.; Hermandeze-Beriain, J. B.; Orengo, J. C.; Saavedra, B.; DeLafc, C.; Fernandez, A. and Boveda, J. B.(2005). Relationship between eheumatoid arthritis and *Mycoplasma pneumonia*e: a case-control study, Rheumatology, 44:912-914.

14- Aletaha D, Neogi T, Silman AJ, *et al.* (2010). Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative . *Ann. Rheum. Dis.* **69** (9): 1580–8.

15- Waites, K. B. and Talkington, D. F. (2004) . *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev. 17:697–728.

16- Kurata1, S. ; Osaki, T.; Yonezawa1, H.; Ken Arae, K.; Haruhiko Taguchi, H. and Kamiya, S. (2014). Role IL-17A and IL-10 in the antigen induced inflammation model by *Mycoplasma pneumoniae*. BMC Microbiology , 14:156

17- Wu, Q. ; Martin, R. J. ; Rino, J. G. ; Breed, R. ; Torres, R. M. and Chu, H. W. (2007) IL-23-dependent IL-17 production isessential in neutrophil recruitment and activity in mouse lung defense against respiratory*Mycoplasma pneumoniae* infection. Microbes.Infect, 9:78–86.

18- Kolls, J. K.; Lindén, A. (2004) Interleukin-17 family members and inflammation. *Immunity* , **21:**467-476.