Chlamydia and *Mycoplasma* serology in respiratory tract infections of children

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ÖZET

Çocuklardaki solunum yolu infeksiyonlarında Chlamydia ve Mycoplasma serolojisi

Çocuklardaki solunum yolu infeksiyonlarının tedavisinin planlanmasında en çok tartışılan konulardan biri de etken olan ajanın tespitidir. Bu çalışmanın amacı, çocuklardaki solunum yolu infeksiyonlarının etyolojisinde Mycoplasma ve Chlamydia sıklığının belirlenmesidir. Bu çalışmaya, yaşları üç ay-12 yaş arasında olan ateş, öksürük, solunum sıkıntısı gibi solunum sistemi semptomları ile pediatri polikliniğine başvuran 100 çocuk dahil edildi. Ayrıntılı klinik öykü alınmasını ve fizik incelemeyi takiben, her hastadan tam kan sayımı, eritrosit sedimentasyon hızı, periferik kan yayması ve akciğer filmi elde edildi. Başvuru anında Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia trachomatis ve Chlamydia psittaci için IgG ve IgM düzeyleri serolojik olarak belirlendi. Chlamydia ve Mycoplasma için pozitif antikor yanıtları 18 (%18) hastada saptandı. Hastaların %2'sinde akut C. pneumoniae infeksiyonu mevcuttu. Geçmişte infeksiyon geçirenler veya tekrar infekte olanlar araştırıldığında; %6 olgunun C. pneumoniae, %3 olgunun C. trachomatis, %1 olgunun C. psittaci ve %8'inin M. pneumoniae ile infekte olduğu saptandı. Eozinofili (≥ %4) veya evde kardeş varlığı klamidyal infeksiyonlar için yatkınlık sağlayan faktörler olarak belirlendi. M. pneumoniae ve C. pneumoniae için yüksek antikor titreleri, iki yaş üzerindeki çocuklarda daha sıklıkla mevcuttu. Alt solunum yolu infeksiyonu olan iki yaş üzerindeki hastalar, atipik ajanlara yönelik antibiyotik tedavileri açısından dikkatle değerlendirilmelidir.

Anahtar Kelimeler: *Mycoplasma, Chlamydia, seroloji, çocuk.*

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SUMMARY

Chlamydia and Mycoplasma serology in respiratory tract infections of children

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One of the challenges in planning the treatment of respiratory tract infection in children is identifying the causative agent. The objective of the present study was to investigate the incidence of Mycoplasma and Chlamydia in the etiology of respiratory tract infections of children. The present study included 100 children, three months to 12 years of age, admitted to the outpatient department of pediatrics with such respiratory symptoms as fever, cough and respiratory distress. Following a detailed clinical history and physical examination, complete blood count, erythrocyte sedimentation rate, peripheral blood smear and chest X-ray were obtained from each patient. At admission, IgG and IgM for Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia trachomatis and Chlamydia psittaci were determined serologically. Positive antibody titer was found for Chlamydia and Mycoplasma in 18 (18%) of the patients. It was found that 2% of the patients had acute C. pneumoniae infection. When the subjects who had infections in the past or had re-infection were also considered; 6% were infected with C. pneumoniae, 3% with C. trachomatis, 1% with C. psittaci and 8% with M. pneumoniae. The presence of eosinophilia (≥ 4%) or the presence of siblings in the house were considered as factors favoring Chlamydial infections. High antibody titers for M. pneumoniae and C. pneumoniae were found more frequently after the age of two. Patients older than two years should be evaluated carefully for antibiotic treatments against atypical agents in pediatric lower respiratory tract infections.

Key Words: *Mycoplasma, chlamydia, serology, children.*

One of the main difficulties in lower respiratory tract infections in pediatric age group is identifying the agent correctly. Culture, antigen screening and serological methods can be helpful in about 1/3 of the cases (1). Atypical pathogens are one of the major causes of pneumonia and may lead to clinical pictures ranging from a mild infectious process to a severe respiratory infection. Although *Chlamydia* and *Mycoplasma* are the most common pathogens, other atypical agents may cause infection. Both pathogens may cause such respiratory tract infections as rhinitis, pharyngitis, bronchitis and pneumonia (2).

Microimmunofluorescence (MIF) test is useful in diagnosing *Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia trachomatis* and *Chlamydia psittaci* infections (3-6). The best diagnostic criteria in an acute infection with these agents are a fourfold rise in antibody titer with the presence of positive polymerase chain reaction (PCR) or growth in culture. While the presence of IgM in serum indicates recent infection, high titers of IgG or IgA indicate ongoing or recurrent infections. Obtaining consecutive serum samples may be required unless PCR or culture is available. Cell culture is very valuable in demonstrating the presence of infection; however, isolating and cultivating the microorganism is very difficult (3). Although MIF test can be utilized in the diagnosing Chlamydial infections, the most important problem is encountered in interpretation (7).

The present study assesses the prevalence of *C. pneumoniae* and *M. pneumoniae* organisms in children between three months and 12 years of age who have been followed in our outpatient department for respiratory tract infection and the relationship between the prevalence of these agents and some clinical and laboratory parameters of the patients.

MATERIALS and METHODS

The study included 100 children between three months and 12 years of age, who presented with

such respiratory symptoms as fever, cough and respiratory distress. Those who have received respiratory support, had previously documented immune deficiencies or a history of hospitalization over the last month prior to the study, underwent surgical operations under general anesthesia, received blood transfusions or immunosuppressive treatment or had known rheumatologic disorders or autoimmune diseases were excluded from the study.

Chronological age, number of siblings, family history of allergy, symptoms of fever, respiratory distress, cough, any antibiotic received at the time of presentation and duration of the symptoms were investigated from the history of the patients. Pulmonary auscultation findings of each patient were recorded during a detailed physical examination.

Venous blood samples were obtained from the patients to perform complete blood count (CBC), differential leukocyte count and erythrocyte sedimentation rate (ESR), and to separate serum for serological studies. Serum samples were stored at -20°C until analysis. IgG and IgM against *C. pneumoniae*, *C. trachomatis*, *C. psittaci* and *M. pneumoniae* were studied using MIF technique (*M. pneumoniae* IgG Antibody Test System and *M. pneumoniae* IgM Antibody Test System; Zeus Scientific Inc., New Jersey, USA, *C. pneumoniae* MIF IgG Test- *C. pneumoniae* MIF IgM Test; Orgenium Laboratories, Turku, Finland). Threshold values that were considered as significant in evaluating the antibody responses of the subjects in the study were as follows: titers above 1/16 for IgM against *C. pneumoniae*, *C. trachomatis*, *C. psittaci* and *M. pneumoniae*; titers above 1/64 for IgG against *C. pneumoniae, C. trachomatis,* and *C. psittaci;* titers above 1/128 for IgG against *M. pneumoniae*.

Statistical Analyses

SPSS software (8.0 version) was used for statistical analyses. The rates of the groups were compared using Pearson's chi-square test and then Fisher's exact chi-square test if the expected values were small. A $p < 0.05$ was considered statistically significant.

RESULTS

The mean age of the patients were 4.5 ± 3.43 years. Thirty-two children (32%) were between ages three months-two years; 21 children (21%), two-four years, 25 children (25%), four-eight years, and 22 children (22%), 8-12 years, respectively. Although there was no significant difference between groups for the numbers of male and female patients, total number of the patients were found to differ among the groups ($p < 0.05$).

C. pneumoniae IgM, *C. trachomatis* IgM, *C. psittaci* IgM and *M. pneumoniae* IgM were found positive in 2 (2%), 3 (3%), 1 (1%) and 5 (5%) of the patients respectively. *C. trachomatis* IgG and *C. psittaci* IgG were not found positive in any of the patients. *C. pneumoniae* IgG and *M. pneumoniae* IgG were found positive in 4 (4%) and 6 (6%) of the patients respectively. In three of the patients; both *M. pneumoniae* IgM and IgG were positive. No significant difference was found between antibody titers of male and female patients. Table 1 shows the antibody titers according to age groups. *M. pneumoniae* and Chlamydial antibody positivity was more common in patients older than two years ($p < 0.05$).

Table 1. Antibody positivity according to age groups.

High antibody titers	Three months-two years (n= 32)	Two-four years (n= 21)	Four-eight years (n= 25)	Eight-twenty years (n= 22)
C. pneumoniae IgM	0 (0%)	1 (1%)	0 (0%)	1 (1%)
C. pneumoniae IgG	0 (0%)	0 (0%)	0 (0%)	4 (4%)
C. pneumoniae, C. trachomatis, C. psittaci IgM + IgG	2 (2%)	1 (1%)	1 (1%)	6 (6%)
M. pneumoniae IgM	0 (0%)	2 (2%)	2 (2%)	1 (1%)
M. pneumoniae IgG	0 (0%)	3 (3%)	3 (3%)	0 (0%)

No significant relation was found among blood hemoglobin, leukocyte counts and differential, ESR and Chlamydial and *M. pneumoniae* antibody titers of the patients in the study. When all Chlamydial antibody titers and eosinophil counts were evaluated, 7 (7.7%) of the patients with eosinophil rates below 4% whereas 3 (33.3%) of the patients with eosinophil rates above 4% were found to have positive antibody response. Thus, a significant relationship was found between increased eosinophil rates and overall Chlamydial antibody titer ($p< 0.05$).

Fifty-nine (59%) patients had fever, 76 (76%) patients had respiratory distress while all patients had cough complaints. No significant correlation was found between a significantly positive antibody titer and the presence of fever. When Chlamydial and *M. pneumoniae* antibody titers were evaluated together, no significant relation was found between positive antibody titer and presence of respiratory distress.

Considering the pulmonary auscultation findings of the patients, crackles and rhonchi were present in 39% and 68% of the patients, respectively. A significant difference was not found between the pulmonary auscultation findings of the patients according to the antibody titers.

Thirty-seven patients (37%) had no sibling, 56 patients (56%) had one sibling, six patients (6%) had two siblings and one patient had more than two siblings. No significant relationship was found between *M. pneumoniae* and Chlamydial antibody titer and number of the siblings. A significant difference was found between those patients with and without siblings for *C. pneumoniae* IgG and overall Chlamydial antibody titer ($p< 0.05$).

Forty-nine patients (49%) had a history of allergy or a positive family history for allergy. Positive antibody titer was found in 10 (25%) of these patients whereas in only eight (13.3%) of the patients without a history of allergy, but the difference was not significant.

The history of the patients revealed that 77 patients (77%) had received antibiotics before admission and 17 (22%) of these antibiotic treatments were against *C. pneumoniae* and *M. pneumoniae*. Positive antibody titer was found in one of these patients (5.9%) and in 17 (20.5%) of the patients who hadn't received antibiotic treatment. No difference was found between antibiotic treatment given to the patients considering the atypical pathogens and the antibody titers. Eighty-five (85%) of the patients were given antibiotic treatment whereas 15 patients (15%) were not given such treatment following presentation. Regarding the use of antibiotic treatment against atypical pathogens, 29 patients had used these antibiotics and positive antibody titer was found in six (20.7%), while in 12 (16.9%) of 71 patients who had not received such antibiotics.

In chest X-ray, infiltration was present in 63% and hyperaeration was present in 20% of the patients. When the Chlamydial and *M. pneumoniae* antibody responses were evaluated together; positive antibody titer was found in 17.5% of the patients with infiltration, in 18.9% of the patients without infiltration, in 10% of the patients with hyperaeration, and in 20% of the patients without hyperaeration. Chlamydial antibody titer was found in 5 (23.8%) of 21 patients with unrevealing chest X-ray whereas *C. pneumoniae* IgM positive antibody titer was found in only two (9.5%) of these patients. No correlation was found between pulmonary radiological findings and *M. pneumoniae* and Chlamydial antibody positivity.

DISCUSSION

Atypical pathogens are one of the most important causes of pneumonia. The diagnosis is usually based on clinical findings, high acute antibody titers and serum antibody tests performed on consecutive blood samples taken with intervals of at least two weeks (2). The most commonly used test in diagnosis of *C. pneumoniae* is MIF. It is considered as "gold standard" in the diagnosis of acute infections because of high sensitivity and specificity (8). Antibiotics in macrolide group are quite effective in treatment of these children and provide a quick improvement (9).

Roles of *M. pneumoniae* and *C. pneumoniae* in 613 children 2 to 14 years old who were hospitalized because of community acquired lower respiratory tract infections were evaluated in a study conducted in Italy (10). Antibody titers were evaluated at the time of admission and four to six weeks later and additionally, nasopharyngeal swab cultures were taken at the time of admission. Acute *M. pneumoniae* infection

was found in 34.3% of the patients while acute *C. pneumoniae* infection was found in 14.1% of them. It was suggested that complications might arise unless the children in this age group are appropriately treated.

Another study evaluated 1104 children with acute lower respiratory tract infections (11). One hundred and fourty-nine patients (13.5%) had acute *C. pneumoniae* infections, 118 patients (10.7%), acute *M. pneumoniae* infections and 27 patients (2.4%), both infections. *M. pneumoniae* was more common than *C. pneumoniae* among the patients with pneumonia whereas *C. pneumoniae* was more common among the patients with bronchitis. Furthermore, it was more common among younger children and those who presented with wheezing. In the present study, no difference was detected in Chlamydial and *M. pneumoniae* antibody responses among the patients with respiratory distress and wheezing.

In the present study, positive antibody titers were found in 3% for *C. trachomatis*, 1% for *C. psittaci*, 6% for *C. pneumoniae* and in 8% for *M. pneumoniae*. This rate was found to be 18% when Chlamydial and *M. pneumoniae* antibody titers were evaluated together. The rates in the present study were lower than previously reported studies. This was considered to be influenced by ethnic differences as well as the fact that only one serum sample had been evaluated at the time of diagnosis. A significant rise was found in *M. pneumoniae* and Chlamydial antibody titers together in the patients older than two years old. This indicates that drug use against atypical pathogens should be considered in the patients older than two years old.

Atypical pneumonia is distinguished from other types of pneumonias by its characteristics such as its insidious and slow course, its ability to lead to different symptoms and complaints in different people and its mild clinical course (12). A study reported the most common findings of atypical pneumonia as fever (92%), cough (52%), leukocyte count above 10.000/mm^3 (56%) and bilateral pulmonary infiltration (60%) (13). Our study didn't show a significant relationship between the patients with and without serological antibody positivity for clinical, radiological and some of the laboratory parameters. Chlamydial antibody titer was found to be statistically higher in the patients with eosinophils above 4%.

In our study positive antibody titer was found for *Chlamydia* and *Mycoplasma* in 18% of the patients. Crowding in family and the presence of eosinophilia might be considered as risk factors for Chlamydial infections.

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