Prevalence of *Mycoplasma pneumoniae* from Symptomatic Pediatric Patients Referred to a Child Outpatient Clinic of a University Hospital

Bir Üniversite Hastanesinde Çocuk Sağlığı ve Hastalıkları Kliniklerine Başvuran Hastalarda *Mycoplasma pneumoniae* Sıklığının Araştırılması

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Abstract

Objective: Lower respiratory tract infections are one of the major causes of morbidity and mortality in children worldwide. Besides, epidemiological data on this subject is very limited. *Mycoplasma pneumoniae* is an important bacterial agent in community-acquired pneumonia (CAP) and may cause mild, moderate and severe lower respiratory tract infections. Clinical diagnosis is very difficult.

Material and Methods: In this study, it was aimed to determine the seroprevalence of *M. pneumoniae* in children aged 0-17 years admitted to a medical faculty in Istanbul with lower respiratory tract complaints or non-respiratory findings such as cytopenia and arthritis/arthralgia and to determine the prevalence of infection among children in various age groups. One hundred and thirty-four patients were included in the present study. Venous blood and nasopharyngeal swab samples were taken from study patients. *M. pneumoniae* IgG and IgM antibodies were determined by ELISA (Enzyme Liquid Immunoassay) test in blood samples and *M. pneumoniae* Real-time polymerase chain reaction (RT-PCR) test was performed in nasopharengeal swab samples.

Results: Seropositivity was seen in 50 (37.3%) patients, only specific IgM antibody positivity in 5 (3.7%) patients, both specific IgM antibody and specific IgG antibody positivity in 8 (6%) patients and 37 (27.6%) patients specific IgG antibody was also observed. The number of *M. pneumoni-*

Öz

Giriş: Alt solunum yolu enfeksiyonları tüm dünyada çocuklarda önemli bir morbidite ve mortalite nedeni olup bu konudaki epidemiyolojik veriler oldukça sınırlıdır. *Mycoplasma pneumoniae*, toplum kökenli pnömonilerde (TKP) önemli bir bakteriyel bir ajandır ve hafif, orta ve ciddi alt solunum yolu enfeksiyonuna neden olabilir. Klinik olarak tanı konması oldukça zordur.

Gereç ve Yöntemler: Bu çalışmada İstanbul'da bir tıp fakültesine alt solunum yolu enfeksiyonu bulguları veya sitopeni ve artrit/artralji gibi solunum dışı bulgularla başvuran 0-17 yaş arası çocuklarda *M. pneumoniae* seroprevalansını belirlemeyi ve çeşitli yaş gruplarındaki çocuklarda enfeksiyon prevalansının dağılımını tespit etmeyi amaçladık. Çalışmamıza 134 hasta dâhil edilmiştir. Çalışmamıza katılan hastalardan venöz kan ve nazofarengeal sürüntü örnekleri alınmış olup, kan örneklerinde *M. pneumoniae* IgG ve IgM antikorlarının tayini Enzim Likit immunassay (ELISA) testi; nazofarengeal sürüntü örneklerinde ise *M. pneumoniae* gerçek zamanlı polimeraz zincir reaksiyonu (GZ-PZR) testi gerçekleştirilmiştir.

Bulgular: Toplam 50 (%37.3) hastada seropozitiflik görülmüş olup, 5 (%3.7) hastada sadece spesifik IgM antikor pozitifliği, 8 (%6) hastada hem spesifik IgM antikoru hem de spesifik IgG antikoru, 37 (%27.6) hastamızda da spesifik IgG antikoru gözlemlenmiştir. *M. pneumoniae* DNA GZ-PZR pozitif örnek sayımız sadece 2 (%1.5) olarak gösterilmiş olup bu hastaların ikisi de serolojik olarak pozitiflik göstermemiş hastalardır.

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ae DNA RT-PCR positive samples was shown to be only 2 (1.5%), both of which were not serologically positive.

Conclusion: Our results show a significant prevalence of *M. pneumoniae* in children diagnosed with acute respiratory infection. It also highlight the importance of laboratory detection for appropriate and rapid antibiotic treatment.

Keywords: Respiratory tract infection, *Mycoplasma pneumoniae*, ELISA, RT-PCR

Sonuç: Sonuçlarımız, akut solunum yolu enfeksiyonu tanısı konan çocuklarda *M. pneumoniae* sıklığını göstermektedir. Ayrıca uygun ve hızlı antibiyotik tedavisi için laboratuvar tespitinin önemini vurgulamaktadır.

Anahtar Kelimeler: Solunum yolu enfeksiyonu, *Mycoplasma pneumoniae*, ELISA, GZ-PZR

Introduction

Lower respiratory tract infections (LRTI) are one of the leading causes of morbidity and mortality in children (1). Epidemiological data on LRTI agents is considerably limited in particularly developing countries (2). *Mycoplasma pneumoniae* is a bacterial agent with increasing importance in community-acquired pneumonia (CAP) worldwide. Its incubation period may last 1-3 weeks. *M. pneumoniae* infection is transmitted from patients through droplets and usually mild and self-limiting (3). It is a common cause of CAP and other community-acquired lower respiratory tract infections (CAP-LRTI) in especially school-aged children and adolescents (4,5). *M. pneumoniae* may result in mild, moderate or severe acute respiratory tract infections (6). Its clinical symptoms range from mild tracheobronchitis to severe atypical pneumonia cases and may be followed by a wide range of extrapulmonary complication spectrum (7). Accurate and rapid detection of the infection is of critical significance in initiating appropriate treatment.

It is difficult to clinically confirm *M. pneumoniae* infection since it is nearly impossible to diagnose this disease based only on clinical signs and symptoms. Therefore, accurate and true diagnosis in order to provide for definitive diagnosis and appropriate treatment and decrease unnecessary antibiotic use of the infected patients is of utmost importance. Laboratory diagnosis of *M. pneumoniae* infections is substantially difficult both because of the requirement to provide special culture conditions for the growth of the agent and the incubation period takes several weeks, which makes clinical practice impossible. Although serological diagnosis is widely used, it can be confusing due to the fact that it gives false negative results particularly in the early acute phase of the infection and serums of the convalescent period cannot be obtained in hospitalizations less than 1 week. The gold standard in the diagnosis of *M. pneumoniae* infections is still a fourfold increase in the antibody titer of sera taken during acute and convalescent periods. (8). Besides, convalescent serum is not useful in clinical practice and does not allow for the clinicians to initiate treatment protocols on time.

Polymerase chain reaction (PCR) stands out as an alternative diagnosis method for etiological agents that are difficult to culture or detect with other methods. PCR is a relatively sensitive and specific diagnostic method for the diagnosis of acute *M. pneumoniae* infections and is used to avoid the risk of false negative results observed in conventional culture methods (9-11). Combination of PCR and serology is recommended for a safer diagnostic approach. Real-time PCR (RT-PCR) is an accurate, efficient and time-saving method for clinicians due to its very fast results, high specificity and sensitivity. In this study, it was aimed to determine *M. pneumoniae* seroprevalence in children aged 0-17 presenting to a Istanbul University Medical School with lower tract respiratory infection symptoms or non-respiratory findings like cytopenia and arthritis/arthralgia and detect the distribution of infection prevalence in children at various age groups.

Materials and Methods

Data of a total of 134 patients presenting to the polyclinics of Pediatric Health and Diseases of Istanbul University Medical School between January 2016 and May 2019 with lower respiratory tract findings or non-respiratory findings like cytopenia and arthritis/arthralgia were retrospectively reviewed. During the acute phase of the disease, venous blood and nasopharyngeal swab specimens were taken from the patients participating in the study. ELISA test was used to determine IgG and IgM antibodies of *M. pneumoniae* in blood samples, and real-time PCR (RT-PCR) was used to study *M. pneumoniae* from nasopharyngeal specimens. Patients in whom only ELISA or RT-PCR tests was performed were excluded from the study.

1-2 mL blood samples taken into dry blood tubes were centrifuged for 5 minutes at 5000 rpm, and suitable samples were included into the study after having checked all for coagulum, hemolysis and lipemia. For *M. pneumoniae* IgM and IgG ELISA test (Vircell, Granada, Spain), patient samples were placed onto Triturus Automatic Microeliza system (Grifols, Barcelona, Spain). Serum samples diluted at a rate of 1:11 with dilution tampon were added to the study well. The study was performed on the Triturus Automatic Microeliza system in line with the manufacturer's instructions. Patient values were read on the spectrophotometer with a 450 nm reference absorbance value, and evaluations were made based on calibrator samples and in line with the manufacturer's instructions. For the presence of *M. pneumoniae* infection, patients with an antibody index of 0.9 or lower and of 1.3 and higher were considered negative and positive respectively, and patients with an index value between 0.9-1.3 were assessed as gray zone.

Table 1. Distribution of clinical findings and laboratory results according to age groups

Findings	IgM	IgM and IgG	IgG	PZR	Negative
Respiratory tract findings					
<5 years	1	1	10	0	29
≥5 years	3	6	13	0	12
Cytopenia					
<5 years	0	0	6	1	24
≥5 years	1	1	7	1	7
Arthritis/Arthralgia					
<5 years	0	0	0	0	2
≥5 years	0	0	1	0	8

Table 2. *M. pneumoniae* seroprevalence according to age and sex

	IgM (+)	IgM (-)	Chi-square	p	IgG (+)	IgG (-)	Chi-square	p
<5 years	2 (7.18)	72 (66.82)	9.24	0.0023	15 (24.85)	59 (49.15)	13.13	0.0002
≥5 years	11 (5.82)	49 (54.18)			30 (20.15)	30 (39.85)		
Girl	10 (5.34)	45 (49.66)	7.65	0.0056	18 (18.47)	37 (36.53)	0.03	0.86
Boy	3 (7.66)	76 (71.34)			27 (26.53)	52 (52.47)		

Nasopharyngeal swab specimens were taken into the transportation liquid (Medical Wire&Equipment, Wiltshire, England). It was provided for the sample material of the samples taken into the transportation liquid with swabs to be dissolved in the transportation liquid, and 400 microliters sample DNA isolation taken from the liquid was achieved in line with the manufacturer's instructions on Qiagen EZ1 Advanced XL automated nucleic acid device (Qiagene, Hilden, Germany) using the Qiagen-EZ1 Virus Mini kit (Qiagene, Hilden, Germany). The DNA samples obtained were applied with RT-PCR on Rotor-Gene Q (Qiagen, Australia) device using the FTD Respiratory Pathogens 21® (Fast-Track Diagnostics, Luxembourg) kit. Some viral agents other than B *M. pneumoniae* can also be detected by this test. 10 µL isolated nucleic acid was put into the 15 µL reaction mixture. RT-PCR condition is 10 minutes at 50°C, 1 minute at 94°C, 8 seconds at 40 cycles 94°C, and 1 minute at 60°C. RT-PCR was performed according to the manufacturer's instructions.

Statistical analyses were done on SPSS (Statistical Package for the Social Sciences) 21.0 program. In order to test the rate difference between qualitative variables, Chi-square test and Fisher's Exact test were used. Statistical significance was set at P< 0.05.

Results

Seventy-four (55.2%) of a total of 134 patients included in our study comprised of children younger than 5 years. Our sample group included 80 (59.7%) boys and 54 (40.3%) girls. In 34 (25.4%) of the 75 patients detected to have lower respiratory tract infection as a result of hospital presentation, IgM (n= 4), IgG (n= 22) or IgM and IgG (n= 7) positivity was deter-

mined. In 17 (12.7%) of the 48 patients detected to have cytopenia, IgM (n= 1), IgG (n= 13), IgM and IgG (n= 1) or RT-PCR positivity was confirmed. In 11 (0.7%) patients detected having arthritis/arthralgia, IgG positivity was detected (Table 1). When serologic findings of the cases were evaluated, seropositivity was seen in 50 patients (37.3%), specific IgM antibody positivity, specific IgM and specific IgG antibody, and specific IgG antibody were observed in 5 (3.7%), 8 (6%) and 37 (27.6%) patients, respectively. Positive number of *M. pneumoniae* RT-PCR cases was only 2 (1.5%), and both of these patients did not show positivity serologically. *M. pneumoniae* RT-PCR result was obtained negative in 5 patients whose specific IgM antibody was positive. Two of the patients with *M. pneumoniae* IgM positivity were aged under 5 years and 11 of them were aged over 5. When children aged under and over 5 years were compared, a significant difference was achieved in terms of *M. pneumoniae* infection (p= 0.002). Moreover, when significance was compared depending on the sexes, a significant difference was not found in the general prevalence; however, *M. pneumoniae* IgM antibodies were found significantly higher in girls (p= 0.004) (Table 2). It was observed based on our study group that the number of cases with suspected.

Discussion

M. pneumoniae pneumonia is observed worldwide, and this agent is responsible for 10-40% of community-acquired pneumonia cases. Nonetheless, information on the epidemiology of these bacteria in Turkey is still limited. In this study conducted to determine prevalence at the age range of 0-17 years, *M. pneumoniae* antibodies were detected in 37.3% of the blood samples. In two different studies carried out on children aged under 15 years in Istanbul, *M. pneumoniae* pos-
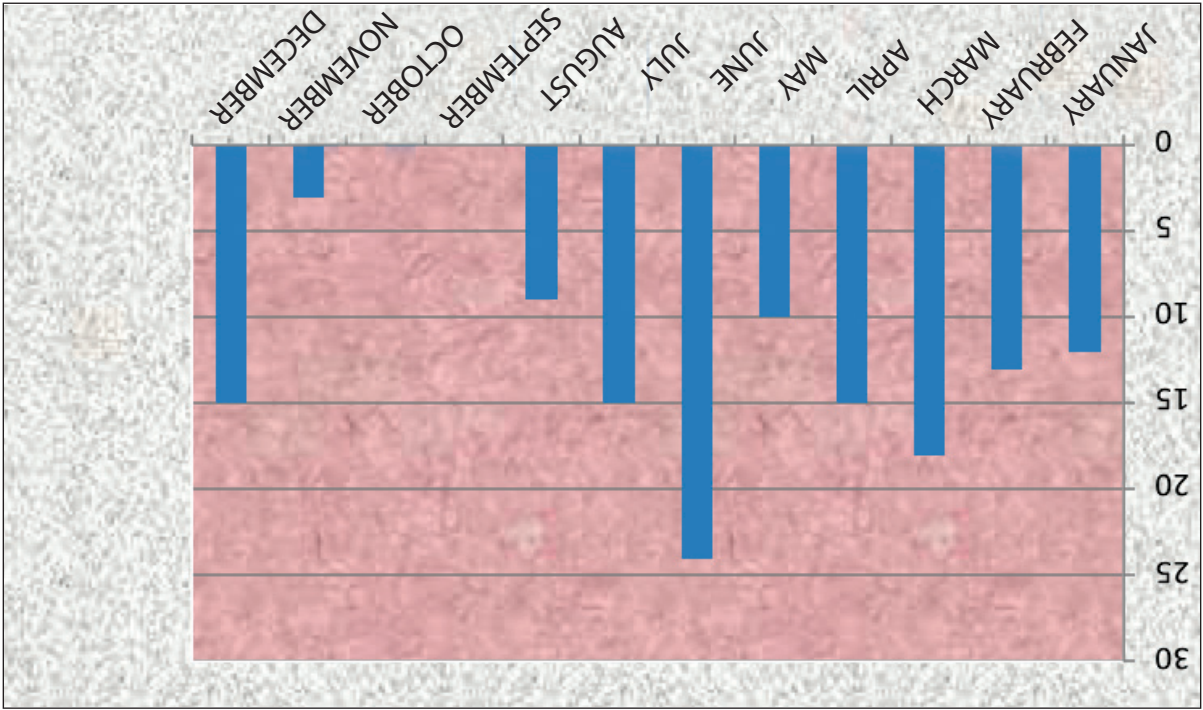
itivity has been reported as 27% and 30% (12,13). In a study conducted in Diyarbakır in 2000, general prevalence was established as 27%(14). Similar results have been observed in a study by Kumar and colleagues (2011), and *M. pneumoniae* serology has been reported positive in 34% of community-acquired LRTI in children (8).

Compatible with previous studies (15,16), our study also pointed out that *M. pneumoniae* prevalence is higher and more significant in children aged over 5 years. This result has been supported by a study conducted on community-acquired pneumonia in recent years. In this study by Jain et al., *M. pneumoniae* has been detected more frequently in children aged ≥5 years (19%) compared to those aged <5 (3%) (17). Similar to studies by Vervloet and colleagues (18) and Heiskanen-Kosma and colleagues (19). *M. pneumoniae* positivity rate was found slightly higher in girls compared to boys in our study; however, a significant relation was not established between the sex of the patient and the incidence of *M. pneumoniae* infection.

Seasonality may not be an important factor in regions where *M. pneumoniae* is endemic; however, more cases have been reported in summer or early fall when epidemics occur (20). In our study group, it was observed that the number of cases with suspected *M. pneumoniae* increased in summer months.

Serology is more sensitive than culture in detecting *M. pneumoniae* acute infection, and comparing PCR techniques with culture and/or serology may not always yield compatible results. In order to diagnose *M. pneumoniae* pneumonia in children, a combination of PCR and acute phase serum immunoglobulin IgM test is the most sensitive and fastest method (21,22). It has been reported that IgM, IgG and IgA could be detected by ELISA in single serum samples of asymptomatic *M. pneumoniae* PCR-positive children. This antibody response in children may reflect a prior encountering with *M. pneumoniae* (23). Generally, a single diagnostic test or combination of tests does not have a differentiating quality of the carrier in a relevant time frame clinically. Positive PCR results in cases of negative serology may be due to insufficient immune response, early successful antibiotic treatment, collection of the samples before specific antibody synthesis, false positive PCR result, carrier without systemic antibody response or false-negative serology tests despite active infection. Negative PCR in serologically-confirmed infections can be related to PCR inhibitors, technical problems with the relevant test or targeted gene and also antibiotic use (24). However, since there are no tests to be used as reference in the diagnosis of Mycoplasma infection, serological analysis for *M. pneumoniae* should be evaluated as standard diagnostic with *M. pneumoniae* specific PCR use. PCR, together with serology, can be used as a good screening test enabling accurate and reliable diagnosis of *M. pneumoniae*.

Current guidelines recommend PCR and serologic tests in order to diagnose *M. pneumoniae* infections (25). PCR is accepted the new "gold standard" which has a higher sensitivity and

Figure 1. Case distribution according to months.

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shorter time frame than culture (26). As in many other respiratory pathogens, *M. pneumoniae* can be carried in the upper respiratory tract of asymptomatic children. Detection rates in children without symptoms of respiratory tract infection changes between 3-56%, which implies that the presence of *M. pneumoniae* alone may not show definite respiratory tract diseases in respiratory tract infections (17,21,23).

The gold standard method of *M. pneumoniae* infection is the fourfold increase in *M. pneumoniae* specific IgG related to the comparison of acute phase and convalescent serums collected with 2-4 weeks interval. The need for convalescent serum sample means that the gold standard can only be used retrospectively and is not useful in clinical practice. Using only acute phase serology seems lacking in sensitivity and specifity. In our study, the low compatibility between PCR and serology may have resulted due to above-mentioned reasons; and moreover, despite being accepted as the appropriate sample, it is seen that nose swab materials and throat swab are insufficient in detecting the agent that locates in lower respiratory tract and causes infection. There are studies on the diagnosis of this agent in bronchoalveolar lavage samples (27). It is evident that studies comparing samples and methods are needed to find the reference standard.

Our results show prevalence of *M. pneumoniae* infections in children diagnosed with acute respiratory tract infection and emphasize the significance of laboratory detection for appropriate, rapid and accurate antibiotic treatment. *M. pneumoniae* is a crucial health problem, especially in children. In our country, limitations in diagnosis in the past had prevented us from understanding the epidemiology of local epidemic environment and spread of this pathogen.

Limited number of cases, retrospective nature of the study, and not having evaluated the amount of antibody increase due to the fact that specific IgG antibodies in the cases were not collected within 2-10 weeks following the increase are the limitations of our study. Nonetheless, the study is significant in investigating regional data aimed at diagnosing *M. pneumoniae* infections.

Conclusion

To conclude, there is no test that definitely differentiates *M. pneumoniae* infection from the carrier or from a prior *M. pneumoniae* infection. Clinician is the person to solve this dilemma. The physician should try to make a diagnosis considering the abovementioned limitations and patients' clinical characteristics. In addition, a national surveillance program should be established for atypical pneumonia etiologies in our country, and RT-PCR use should be evaluated as a reliable diagnostic method with further studies.

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