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Frequency of mycoplasma pneumonia among children hospitalized with community - acquired pneumonia

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Abstract

Introduction: Community-acquired pneumonia (CAP) is one of the most common respiratory infections and is clinically divided into typical and atypical. The most common causal microorganism for typical pneumonia is *Streptococcus pneumoniae*. Conversely, the most frequent microorganisms in typical pneumonia are *Mycoplasma pneumonia* and viruses.
Objectives: The purpose of this study was to investigate the frequency of *M. pneumonia* in children with diagnosis of pneumonia.
Patients and Methods: The present study is a descriptive-analytical study. This study was conducted on 195 children with *M. pneumonia*. The diagnosis was confirmed by clinical features, laboratory data and radiological findings.
Results: In this study, the mean age of patients was 4.89 years since 61.5% of patients were boys. Of them, serum IgMwaspositivein13 patients and IgG was positive in 41 patients. Among the clinical symptoms, fever, cough and runny nose were the most common symptoms. Patients with positive IgM usually were older and had higher fever than other patients while their serum C-reactive protein (CRP) level was significantly higher ($P < 0.05$).
Conclusion: The results of this study showed that frequency of *M. pneumonia* increase with age. High grade fever and sever cough are more common in children with *M. pneumonia*. Among the laboratory findings higher CRP level is reliable predictor marker for *M. pneumonia* infection.

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Introduction

Pneumonia is one of the most common respiratory infections. The infection can even be fatal in children and infants, especially in children with immunodeficiency. According to the World Health Organization (WHO) reports, pneumonia was one of the leading causes of death for children in 2010 (1). Pneumonia accounts for 21% of the under five years old mortality rate in Africa and the Eastern Mediterranean and 12% in American and European countries. As studies have been conducted in East Asia; for example in 2012 in Taiwan, pneumonia was the fifth leading cause of death among children aged 1 to 4 years (2). Community-acquired pneumonia (CAP) is also one of the most common causes of hospitalization in children. The CAP is often clinically divided into typical and atypical. Typical pneumonia is caused by Streptococcus pneumoniae and atypical pneumonia is caused by microorganisms such as *Mycoplasma pneumonia* and viruses (1).

Mycoplasma pneumoniae is one of the most common pathogens causing pneumonia, especially in school-age children. The *M. pneumonia* infection occurs in all seasons but is more prevalent in the spring and autumn. Although the most common symptom of *M. pneumonia* infection is cough without a runny nose, but may vary from asymptomatic respiratory infection to serious pneumonia (3). While macrolides and tetracyclines are effective against mycoplasma pneumonia, they are not effective in cases of CAP. *M. pneumonia* often is a fluoroquinolone sensitive microorganism, however it should be administered with caution in children due to side effects and joint toxicity (4).The resistance of this pathogen to macrolides in countries such as China is 90% to 100%

Key point

One of the most frequent microorganisms in atypical pneumonia is *Mycoplasma pneumoniae*. This study investigated the frequency of *M. pneumonia* in children with diagnosis of pneumonia. Patients with positive IgM, usually were older and had higher fever than other patients and their serum CRP level was significantly higher.

and in Taiwan is 10% to 20% (5,6). Therefore, accurate identification methods for *M. pneumonia* infection are required, to avoid the widespread administration of antibiotics and the subsequent development of resistance. The culture of *M. pneumonia* is very difficult and it has a very slow growth rate. Therefore, the culture of this microorganism is not helpful for diagnosis. Now in both adults and infants, the polymerase chain reaction (PCR) is conducted as primary laboratory procedure for *M. pneumonia* respiratory infections diagnosis (7, 8). Sputum sampling is not recommended, since throat sampling in young children is difficult, while studies have shown no significant differences in PCR of infected and healthy individuals (9). Serological tests are the main method for the diagnosis of *M. pneumonia* infections. The complement fixation method is often conducted for this purpose, however due to its complexity and the high number of false positive results of this method, it is not widely recommended (10,11). Some patients may not have adequate levels of *M. pneumonia* immunoglobulin M (IgM) antibody in the early stage of the disease, since the presence of the IgM determines the presence of acute *M. pneumonia* infection(12,13), however a few studies have conducted the immunoglobulin A (IgA) antibody, which is not very sensitive in infants. Therefore, there is great controversy regarding the best method for diagnosis of *M. pneumonia* infection in infants (14). As mentioned above, pneumonia has been one of the main causes of death in children, since the prevalence of *M. pneumonia* is high in the most parts of the world and additionally antibiotic resistance to this microorganism is rising to high levels(15).

Objectives

This study aimed to determine the prevalence of *M. pneumonia* in children with diagnosis of pneumonia.

Patients and Methods

Study design

The present study was a cross-sectional study to determine the frequency of *M. pneumonia* among children with pneumonia who referred to Motahari hospital in Urmia, Iran (2019 to 2020). The sample collecting was conducted by the convenience sampling method. Demographic and clinical information of patients including age, gender and symptoms such as cough, fever, runny nose, vomiting, diarrhea and abdominal pain as well as outcome as discharge or death and radiologic and laboratory findings were extracted from patients' medical records. The diagnosis of *Mycoplasma* infection was based on clinical features, radiological findings and more than four times increase in serum IgG and IgM level. Serum IgG and IgM levels more than 12 ng/mL were considered as positive, while level less than 8 ng/mL was considered negative, since 8-12 ng/mL found as borderline.

The main different patterns of chest-X ray findings in *M. pneumonia* infection consist of peribronchial and perivascular interstitial infiltrates, reticular densities (can be patchy with a segmental or non-segmental distribution), airspace consolidation, reticulonodularo pacification, nodular or mass-like opacification. Besides bilateral peribronchial, perivascular, interstitial infiltrates in central and middle lung zones, bilateral lesions, pleural effusion and hilar lymphadenopathy are the other patterns of chest X-ray presentations in *M. pneumonia*.

The inclusion and exclusion criteria

Children who were hospitalized with pneumonia and *M. pneumonia* confirmed by clinical features, laboratory data and radiological findings enrolled in the study. Exclusion criteria were defined as incomplete medical records, patients with recurrent aspiration pneumonia due to neurological disease, immunocompromised patients and users of immunosuppressant drugs, as well as patients with a history of chronic lung disease.

Serological tests

At the time of admission, one milliliter of blood was drawn from each patient, then the serum was isolated and stored at -70°C for the next antibody test. To detect IgG and IgM antibodies for mycoplasma pneumonia, an enzyme-linked immunosorbent assay (ELISA) was conducted by applying the manufacturer's instructions. Serum IgM and IgG antibody concentrations above 12 U/mL and less than 8 U/mL were considered as positive and negative, respectively. The intermediate result (between 8 and 12 U/mL) was also supposed as negative.

Statistical analysis

Data were analyzed using Statistical Program for the Social Sciences (SPSS) version 22. Descriptive statistics were reported in terms of frequencies or percentages for qualitative data, and for quantitative data in terms of means and standard deviations (SD). Additionally, the Pearson's correlation coefficient, chi-square test and independent *t* test were applied to data analysis. Differences were considered significant at ($P<0.05$). The Kolmogorov-Smirnov test is used to test normality distribution of results. Additionally, a *P* value less than 0.05 was considered as statistically significant.

Results

In the current study, the mean age of 195 children was 4.89± 2.38 years. Among patients with positive IgG levels, the mean age was 5.78 ± 2.42 years. Additionally, the mean age for patients who were IgM positive was 6.45 ± 2.47 years. According to analysis was conducted by Kolmogorov–Smirnov and independent *t* test there was a significant difference between the serum level of children's IgM and IgG in terms of age; therefore, children with positive levels of both IgM and IgG were older than children with negative IgM and IgG levels ($P = 0.01$ and *P*

= 0.03 respectively).

Out of 195 patients, 120 children (61.5%) were boys. There was no significant statistical difference of serum antibody levels in both genders ($P=0.77$ and $P=00.24$ respectively). IgM level were negative and positive in 182 and 13 patients, respectively. Out of 13 positive samples, 4 girls and 9 boys were positive for IgM. IgG level were negative in 154 samples and positive in 41 patients. In our study, twenty of IgG positive patients were girls and 21were boys. In eight patients of both IgM and IgG levels were positive.

The most common symptoms among the 195 patients were cough in 149 cases (87.2%), fever in 137 cases (76.9%), runny nose in 38 cases (25.1%) and nausea and vomiting in 22 cases (12.3 %). Just 36 patients (18.5 %) had radiological findings in their chest X-ray. The most common findings were lobar consolidation (9.2%), pleural effusion (6.2 %) and patchy infiltration (3.1%).

According to the frequency distribution of positive or negative IgM test results, fever and cough were more significant in IgM-positive children ($P=00.042$ and $P=0.03$; Table 1).

The results of the frequency distribution of positive or negative IgG tests have shown no significant difference in terms of clinical complaints and radiological findings between positive and negative IgG patients ($P>0.05$; Table 2).

Our study showed no significant relationship between the two groups of positive and negative IgM and IgG in the complete blood count and erythrocyte sedimentation rate results ($P> 0.05$; independent t test). In the study population, in 98 patients (50.3%) were CRP had less than 6 mg/L. Among the patients who were positive for IgM, only two patients (15.4%) had CRP less than 6 mg/L. Additionally, five patients (38.5%) had CRP between 6 mg/L and 20 mg/L, since three patients (23.1%) had CRP 21 mg/L to 50 mg/L and three patients (23.1%) had CRP more than 50 mg/L. Based on chi-square test, IgM-positive patients had significantly more levels of CRP than IgM-negative patients ($P = 0.03$).

Among patients who were positive for IgG, in 21 patients (51.2%) CRP levels was less than 6. The chi-square test showed no significant difference between positive and negative IgG subjects in terms of CRP ($P = 0.06$).

Discussion

According to the WHO reports, pneumonia is responsible for 21% of children deaths in Africa and in the Eastern Mediterranean and 12% in the United States and Europe. More than 40% of CAP in children is caused by *M.*

Table 1. Comparison of positive and negative IgM groups in terms of clinical complaints and radiological findings

Complaints and findings	Group				*P* value
	IgM-positive		IgM-negative		
	No.	%	No.	%	
Fever	13	100	137	75.3	0.02
Cough	12	92.3	149	81.9	0.03
Runny nose	2	15.4	47	25.8	0.32
Nausea and vomiting	2	15.4	22	12.1	0.49
Abdominal pain	0	0	18	9.9	0.27
Diarrhea	2	15.4	10	5.5	0.18
Anorexia	1	7.7	8	4.4	0.47
Consolidation	1	7.7	17	9.3	
Pleural effusion	1	7.7	11	6	0.91
Patchy infiltrate	0	-	6	3.3	

Table 2. Comparison of positive and negative IgG groups in terms of clinical complaints and radiological findings

Complaints and findings	Group				P value
	IgG-positive		IgG-negative		
	No.	%	No.	%	
Fever	31	75.6	119	77.3	0.83
Cough	30	73.2	131	85.1	0.1
Runny nose	11	26.8	38	24.7	0.84
Nausea and vomiting	7	17.1	17	11	0.29
Abdominal pain	6	14.6	12	7.8	0.22
Diarrhea	5	12.2	7	4.5	0.13
Anorexia	1	2.4	8	5.2	0.68
Consolidation	7	17.1	11	7.1	
Pleural effusion	1	2.4	11	7.1	0.14
Patchy infiltrate	2	9.4	4	2.6	

pneumonia and approximately 18% of *M. pneumonia* patients need hospitalization (17). Mycoplasma pneumonia is a small bacterium without a cell wall that making it resistant to many antibiotics, including beta-lactams. The most common symptoms are fever and cough, but extra-pulmonary symptoms may also occur. This microorganism usually affects the upper airways in children under the age of three, while bronchitis is more common in people aged five to twenty (18).

In a study by Medjo et al (16) in Serbia, out of 166 children with pneumonia, 24 patients (14.5 %) had *M. pneumonia* which is more than the prevalence calculated in our study. The reason for this can be due to the use of different tests with different sensitivity in two studies, in the study of Medjo et al, PCR tests and serological tests were used.

Additionally, in the study of Defilippi et al which PCR test was the diagnostic tool, the prevalence of *M. pneumonia* was higher than our study (12%) (19). However, 63 patients (7.13%) of the total study population had a positive serological test, which is close to the prevalence values in our study. In a study by Almasri et al in Greek, 25.1% of patients had IgG- positive test for *M. pneumonia*, which is similar to our findings and none of the patients in this sample were IgM positive (20). However, in the study of Ma et al, 15.04% of patients had positive IgM, which is higher than the recent study (1).

There was no significant difference in IgG and IgM antibodies level in both genders. Similar to results of Medjo et al and Tonella et al, almost patients with *M. pneumonia* were male (16,21).

The mean age of patients was 4.89 years. There was a statistically significant difference between the serum level of IgM and IgG in terms of age. In other words, patients with positive IgG and IgM were older than others (P = 0.02, P = 0.01, respectively) (16). In the study by Defilippi et al, most of patients with *M. pneumonia* infection were among 6 and 7 years, which is similar to the mean age of patients with positive IgM in our study (19).

Several studies have shown that in school and pre-school age, *M. pneumonia* infection is more prevalent (1,16,19). The explanation for the higher prevalence of this infection in older ages may be attributed to increased exposure of children to this microorganism as a result of their increased social activities.

In the current study, cough, fever and runny nose were the most common clinical symptoms like as other investigations. But gastrointestinal symptoms were more prominent in other studies. As our analysis in IgM positive patients cough and fever was more prominent than IgM negative group.

As results patients with *M. pneumonia* (IgM positive) had statistically significant higher positive rate of CRP test than other patients, it was like as the study of Hsieh et al that 72% of patients had a positive CRP test (23).

In contrast to our results in the study of Medjo et al the rate of radiological findings was higher (16). This difference may be due to differences in the timing of the imaging, technique, and operator-dependent interpretation.

Conclusion

As our results *M. pneumonia* is more prevalent in school-age and among the radiological and laboratory findings, only CRP was correlated with infection of this atypical bacterial strain.

Limitations of the study

PCR is one of the gold standard tests for diagnosis of mycoplasma infection, which was unavailable in our center.

Authors' contribution

NA and SE conceptualized the presented idea. NA, FG and SE were the principal investigators of the study. FG, KM included in preparing the concept and method. KM developed the theory and performed the computations. FG and SE verified the analytical methods. NA supervised the findings of this work. All authors participated in visualization of study and preparing the original draft of the manuscript, review and editing the manuscript and critically evaluated the intellectual contents. Provision of study material, patients and laboratory samples was done by KM Data curation and project administration was done by FG In this study there was no need to funding acquisition. All authors discussed the results and contributed to the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Urmia University of Medical Sciences approved this study (IR-UMSU.REC.1398.375). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from M.D., thesis of Marzieh Khaneshi at this university (Thesis#99.9829). Besides, ethical issues (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

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References

1. Ma YJ, Wang SM, Cho YH, Shen CF, Liu CC, Chi H, et al. Clinical and epidemiological characteristics in children with community-acquired mycoplasma pneumonia in Taiwan: A nationwide surveillance. J Microbiol Immunol Infect. 2015;48:632-8. doi: 10.1016/j.jmii.2014.08.003.
2. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ. 2008;86:408-16. doi: 10.2471/blt.07.048769.
3. Ali NJ, Sillis M, Andrews BE, Jenkins PF, Harrison BD. The clinical spectrum and diagnosis of Mycoplasma pneumoniae infection. Q J Med. 1986;58:241-51.
4. Bradley JS, Jackson MA; Committee on Infectious Diseases; American Academy of Pediatrics. The use of systemic and topical fluoroquinolones. Pediatrics. 2011;128:e1034-45. doi: 10.1542/peds.2011-1496.
5. Pereyre S, Goret J, Bébéar C. Mycoplasma pneumoniae:

Current Knowledge on Macrolide Resistance and Treatment. Front Microbiol. 2016;7:974. doi: 10.3389/fmicb.2016.00974.
6. Chen YC, Hsu WY, Chang TH. Macrolide-Resistant Mycoplasma pneumoniae Infections in Pediatric Community-Acquired Pneumonia. Emerg Infect Dis. 2020;26:1382-1391. doi: 10.3201/eid2607.200017.
7. Räty R, Rönkkö E, Kleemola M. Sample type is crucial to the diagnosis of Mycoplasma pneumoniae pneumonia by PCR. J Med Microbiol. 2005;54:287-291. doi: 10.1099/jmm.0.45888-0.
8. McCracken GH, Hardy RD. Diagnostic utility and clinical significance of naso- and oropharyngeal samples used in a PCR assay to diagnose Mycoplasma pneumoniae infection in children with community-acquired pneumonia. J Clin Microbiol. 2004;42:3339-41. doi: 10.1128/JCM.42.7.3339-3341.2004.
9. Spuesens EB, Fraaij PL, Visser EG, Hoogenboezem T, Hop WC, van Adrichem LN, et al. Carriage of Mycoplasma pneumoniae in the upper respiratory tract of symptomatic and asymptomatic children: an observational study. PLoS Med. 2013;10:e1001444. doi: 10.1371/journal.pmed.1001444.
10. Pönkä A, Pönkä T, Sarna S, Penttinen K. Questionable specificity of lipid antigen in the Mycoplasma pneumoniae complement fixation test in patients with extrapulmonary manifestations. J Infect. 1981;3:332-8. doi: 10.1016/s0163-4453(81)91901-0.
11. Kleemola M, Käyhty H. Increase in titers of antibodies to Mycoplasma pneumoniae in patients with purulent meningitis. J Infect Dis. 1982;146:284-8. doi: 10.1093/infdis/146.2.284.
12. Sillis M. The limitations of IgM assays in the serological diagnosis of Mycoplasma pneumoniae infections. J Med Microbiol. 1990;33:253-8. doi: 10.1099/00222615-33-4-253.
13. Thacker WL, Talkington DF. Analysis of complement fixation and commercial enzyme immunoassays for detection of antibodies to Mycoplasma pneumoniae in human serum. Clin Diagn Lab Immunol. 2000;7:778-80. doi: 10.1128/cdli.7.5.778-780.2000.
14. Yamazaki T, Narita M, Sasaki N, Kenri T, Arakawa Y, Sasaki T. Comparison of PCR for sputum samples obtained by induced cough and serological tests for diagnosis of Mycoplasma pneumoniae infection in children. Clin Vaccine Immunol. 2006;13:708-10. doi: 10.1128/CVI.00413-05.
15. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev. 2004;17:697-728, table of contents. doi: 10.1128/CMR.17.4.697-728.2004.
16. Medjo B, Atanaskovic-Markovic M, Radic S, Nikolic D, Lukac M, Djukic S,et al. Mycoplasma pneumoniae as a causative agent of community-acquired pneumonia in children: clinical features and laboratory diagnosis. Ital J Pediatr. 2014 Dec 18;40:104. doi: 10.1186/s13052-014-0104-4.
17. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev. 2004;17:697-728, table of contents. doi: 10.1128/CMR.17.4.697-728.2004.
18. Mandell G. Bennett's Principles and Practice of Infectious Diseases. Vol. 7. Philadelphia: Churchill Livingstone/Elsevier. 2010.
19. Defilippi A, Silvestri M, Tacchella A, Giacchino R, Melioli G, Di Marco E, et al. Epidemiology and clinical features of Mycoplasma pneumoniae infection in children. Respir Med. 2008;102:1762-8. doi: 10.1016/j.rmed.2008.06.022.
20. Almasri M, Diza E, Papa A, Eboriadou M, Souliou E. Mycoplasma pneumoniae respiratory tract infections among Greek children. Hippokratia. 2011;15:147-52.
21. Tonella M, Maronati A, Giraldo C, Pozzoni A, Cassani MR, Maltagliati S, et al. (2011). Mycoplasma pneumoniae: IgG and IgM antibody response in presence of different antigens. Evaluation of commercial tests. Microbiol Medica, 26. doi: 10.4081/mm.2011.2342.
22. Lee WJ, Huang EY, Tsai CM, Kuo KC, Huang YC, Hsieh KS, et al. Role of Serum Mycoplasma pneumoniae IgA, IgM, and IgG in the Diagnosis of Mycoplasma pneumoniae-Related Pneumonia in School-Age Children and Adolescents. Clin Vaccine Immunol. 2017 Jan 5;24:e00471-16. doi: 10.1128/CVI.00471-16.
23. Hsieh SC, Kuo YT, Chern MS, Chen CY, Chan WP, Yu C, et al. Mycoplasma pneumonia: clinical and radiographic features in 39 children. Pediatr Int. 2007;49:363-7. doi: 10.1111/j.1442-200X.2007.02363.x. PMID: 17532837.