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Original Article

A study of prevalence of *Mycoplasma pneumoniae* pneumonia and validation of Immunoglobulin M for *Mycoplasma pneumoniae* in the diagnosis of *Mycoplasma pneumoniae* pneumonia

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Abstract

Background: *Mycoplasma pneumoniae* pneumonia (MPP) is one of the most common causes of childhood community-acquired pneumonia (CAP) and common cause of mortality and morbidity in young children. Polymerase chain reaction (PCR) is promising with higher specificity and superior sensitivity to that of culture or single point serology. **Objective:** This study was conducted to estimate the prevalence of MPP, and to compare the efficacy of PCR and immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) for the diagnosis of MPP among CAP in children. **Materials and Methods:** This study was conducted in children between 2 months and 12 years of age admitted with clinical and radiological features of pneumonia. The children were presumed clinically to have MP infection as per IAP guidelines when they had a cough and fever for more than 5 days. A predesigned proforma was used to collect history and clinical examination findings. Routine investigations such as complete blood count, C-reactive protein, chest X-ray, and blood culture were done. Those suspected of infection with MP infection were further subjected to PCR and IgM ELISA. **Results:** MP (27%) was found to be an important cause of CAP in children between 2 and 8 years. The most common clinical symptoms were cough and fever (100%) followed by myalgia (20.8%), arthralgia (16.6%), and rashes (12.5%). PCR is the rapid reliable diagnostic test with a sensitivity of 100% and specificity of 93%. IgM ELISA is equally effective diagnostic test with sensitivity of 83% and specificity of 100%. **Conclusion:** MP is an emerging cause of CAP in school going as well as preschool children. Although PCR is an alternative test of culture, use of simple test like IgM ELISA will reduce the cost of investigation and help us in arriving at definitive diagnosis of MP.

Key words: *Community-acquired pneumonia, Immunoglobulin M enzyme-linked immunosorbent assay, Mycoplasma pneumonia, Polymerase chain reaction*

Mycoplasma pneumonia (MP) is one of the most common causes of childhood community-acquired pneumonia (CAP) and also the common cause of mortality and morbidity in young children [1]. The WHO has estimated that annually, pneumonia kills up to 2.4 million children, which accounts for 19% of all deaths in under-five age group [2]. MP pneumonia (MPP) is a disease of gradual onset associated with fever, cough, headache, myalgia, sore throat, and other nonspecific symptoms. However, these are not specific enough for the diagnosis of MPP in clinical practice. β-lactam antibiotics used empirically in the treatment of pneumonia are ineffective in these cases. Hence, a rapid diagnostic method is required to initiate the appropriate treatment.

Because of the fastidious nature of MP, culture methods are relatively insensitive, time-consuming, and expensive [3]. The commercially available enzyme-linked immunosorbent assay (ELISA) is more specific and sensitive but require paired sera for diagnosis [4]. Polymerase chain reaction (PCR) is promising with higher specificity and superior sensitivity to that of culture or single point serology [5]. Information on diagnostic methods used for MP in Indian pediatric population is scarce [6]. Thus, this study was conducted to know the prevalence of MP and to compare the efficacy of PCR and immunoglobulin M (IgM) ELISA for the diagnosis of MPP among CAP infection in children.

MATERIALS AND METHODS

This study was conducted at Indira Gandhi Institute of Child Health (IGICH), Bangalore, a tertiary care pediatric hospital from November 2012 to September 2013. This study was approved by the Institutional Ethical Committee. All the children in the age group of 2 months to 12 years admitted with a diagnosis of pneumonia during the study period were enrolled. Diagnosis of pneumonia was made on the basis of clinical features of pneumonia as per Integrated Management of Neonatal and Childhood Illness Guidelines as well as clinical

criteria and radiological features for children more than 5 years as per RTI GEM- IAP and Nelson Text Book of Pediatrics [7]. The children less than 2 months of age, who were treated with macrolides in the preceding 14 days, children with predisposing factors for recurrent pneumonia and with chronic lung disease were excluded from the study.

Children were presumed clinically to have MP infection as per IAP guidelines when they had cough and fever for more than 5 days with or without systemic symptoms such as myalgia and arthralgia, hurried breathing and respiratory clinical signs predominantly crepitations, rhonchi without obvious evidence of localized consolidation. The children were enrolled into the study after taking informed consent from the parents.

These children after obtaining detailed history were examined for signs and symptoms of pneumonia, and evaluated for respiratory distress. The oxygen saturation (by pulse oximetry) was also noted. Auscultation findings (crepitations, wheeze, bronchial breath sounds) were recorded. These children were subjected to routine investigations such as complete blood count, C-reactive protein (CRP), chest X-ray, and blood culture and those suspected of infection with MP were further subjected for PCR and IgM ELISA of the same. All Children were given standard treatment for pneumonia and those children who were positive for MP were treated with Macrolides.

ELISA for IgM

Blood was collected from all the suspected 86 cases with clinical features of pneumonia. Serum was separated and stored at -20°C and IgM antibody detection was done using Nova Tec Immunodiagnostica kit GmbH Germany, according to the manufacture's instruction. It is based on the principle that gelatin particles sensitized with membrane components of MP strain are agglutinated in the presence of MP antibody and mainly measure IgM for MP [8].

PCR Methodology

The test was outsourced and done in NABH accredited laboratory. Nasopharyngeal swabs were collected from all the clinically suspected MP cases. After DNA extraction, specific primers were used for priming and amplification which was done in thermocycler. Amplified products were subjected for identification (electrophoresis). The presence of specific amplification band is matched with a standard band of MP.

Statistical Analysis

Descriptive and inferential statistical analysis has been carried out in this study. Results on categorical measurements are presented in number (%). The significance is assessed at 5% level. Chi-square/Fisher exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

RESULTS

Out of total 568 cases admitted with pneumonia, 86 children were clinically suspected of MP. Moreover, out of these 86 cases, PCR was positive in 24 cases accounting for the prevalence of 4.2% among CAP. In our study, among the cohort of PCR confirmed MP, 13 (54%) children were between 2 and 8 years of age. There was male preponderance with male to female ratio of 1.4:1 (Table 1).

We found that all the children had cough, fever, and crepitations. About 45% of these children had hurried breathing and 41% of them had chest retractions. In our cohort of PCR positive MP, anemia was seen in 25% of the children and serum CRP was elevated in 87.5% cases. The radiological findings include reticulonodular infiltrates (62.5%), bilateral peribronchial interstitial infiltrates (16.6%), bronchopneumonia (12.5%), and nonspecific changes (8.3%) (Table 2).

The IgM for MP was positive in 83% of the children with PCR positive MP and none of PCR-negative cases as shown in Table 3. The sensitivity and specificity of IgM MP was 83.3% (95% confidence interval [CI] - 61.8%-94.5%) and 80.6% (95% CI - 68.2%-89.1%), respectively.

DISCUSSION

MP infection is one of the common causes of CAP especially in school going children with reported incidence of 15-30%, in India [9]. The clinical features are nonspecific and confirmatory lab test is PCR which is expensive. Hence, there is need to evaluate a simple and cost-effective lab diagnostic test to confirm the diagnosis. We studied to validate the IgM for MP test to detect the MPP.

In our study, the most common age group affected by MPP was 2-8 years (54.1%) which was in concordance with study conducted by Shenoy et al. [9], Ramamoorthy et al. [10], and Waris et al. [11]. Although in previous studies MPP is found to be common in school age group, the present study has

Table 1: Baseline data

Data (n=24)	Number	Percentage
Age (years)		
<2	5	21
2-8	13	54
More than 8	6	25
Gender		
Male	14	58
Female	10	42

Table 2: Clinical and lab features

Features	Number	Percentage
Cough	24	100
Fever	24	100
Hurried breathing	11	45
Feeding difficulty	06	21
Crepitations	24	100
Retraction	10	41.6
Rhonchi	04	16.6
Myalgia	05	20.7
Arthralgia	04	16.6
Rashes	03	12.5
Chest pain	01	4.6
Lab parameters		
Hb% <10 g/dl	6	25
Hb% >10 m/dl	18	75
Positive CRP	21	87.5
Radiological findings		
Reticulonodular infiltrates	15	62.5
B/L peribronchial interstitial infiltrates	04	16.6
Bronchopneumonia	03	12.5
Nonspecific	02	8.3

Hb: Hemoglobin, CRP: C-reactive protein

Table 3: Correlation of IgM for MP and PCR for MP

IgM	PCR (%)		Total (%)	Chi-square p value
	Positive	Negative		
Positive	20 (83.3)	0 (0)	20 (23.25)	χ^2=62.73
Negative	4 (16.7)	62 (100)	66 (76.75)	p<0.01**
Total	24 (100.0)	62 (100.0)	86 (100)	

IgM: Immunoglobulin M, PCR: Polymerase chain reaction, MP: *Mycoplasma pneumoniae*, ** P value < 0.05 is significant

shown that there is a changing trend of MPP involving even the preschool age group.

In our study, common clinical presentations were cough and fever (100%), and extrapulmonary symptoms such as myalgia (20.8%), arthralgia (16.6%), and rashes (12.5%). This was similar to the study done by Kashyap et al., where fever (100%), cough (100%), and tachypnea (37.2%) were main clinical presentations. The common respiratory signs were crepitations (100%), retractions (41.6%), and rhonchi (16.6%). Kashyap et al., [6] also showed scattered crepitations (78.67%) and rhonchi (37.32%) as the predominant signs. Radiological features found in our study cohort were similar to that shown in a study by Kashyap et al. [6].

Although culture is the gold standard test to confirm MP infection, culture is tedious and difficult to grow the organism. Hence, PCR has been taken as the gold standard method to confirm the diagnosis of MPP [12]. It is time saving and has high sensitivity and specificity as shown in previous studies by Kashyap et al., [6] and Nadala et al. [13]. In our study, PCR was positive in 24 (27.9%) clinically suspected cases which was similar to the observations made by Sidal et al., [14] and Blackmore et al., [15] who found MP PCR positive in 33 (16.2%) and 25 (25%) cases, respectively. The sensitivity and specificity of PCR were 92% and 98%, respectively, in the study by Blackmore et al., [15]and they concluded that PCR appeared to have advantages over serological testing, both with respect to accuracy and convenience of single specimen testing.

In the present study, IgM antibody was positive in 20 (23.9%) out of 86 children with suspected MPP which was in concordance to study done Chaudhry et al. [16]. They found serological evidence of MP in 17 (27.4%) out of 62 children based on microparticle agglutination test for IgM antibodies and indirect immunofluorescence test for antigen detection from throat swabs. Similarly, Pandey et al., [17] showed IgM positivity for MP in 21 (30%) out of 70 children. In a study by Ramamoorthi et al. [10], culture and IgM antibody positivity for MP were 10.5% and 31.5%, respectively. They showed that the diagnosis of MPP may be made routinely in clinical microbiology units using IgM ELISA; however, IgM ELISA was less sensitive in very early infection as compared to PCR.

In our study, 20 (23.9%) out of 86 cases were positive by IgM ELISA with sensitivity of 83% and specificity of 80.6%. Waris et al., [11] showed that, 24 out of 24 MPP children (sensitivity, 100%) were positive by IgM-capture test with convalescent phase serum, 19 (79%) were positive by the IgM- capture test with acute phase serum, 19 (79%) were positive by IgG serology, 10 (41.6%) were positive by PCR and 8 of 17 (47%) were positive by culture. They concluded that IgM serology test was the single most valuable tool for the diagnosis of MPP in children of any age.

The detection of MP in symptomatic children by specific IgM detections was the optimum approach for the diagnosis in our study. The advantage of this being easy and early diagnosis of MPP so as to decrease the duration of hospital stay as well as cost of treatment considerably using simple antibiotics like macrolides and avoiding unnecessary inappropriate irrational antibiotic usage. However, our study was limited by small sample size and it needs a larger group to arrive at a definitive conclusion.

CONCLUSION

The prevalence of MP among CAP was 4.2%, and the majority of them was in children between 2 and 8 years of age. The sensitivity and specificity of IgM for MP was 83.3% and 80.6%, respectively. For optimal diagnosis and timely initiation of therapy, the combination of clinical features, PCR and IgM for MP may be more beneficial.

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