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CLINICAL SPECTRUM AND DETECTION OF MYCOPLASMA PNEUMONIAE IN INFANTS WITH WHEEZE IN COMMUNITY ACQUIRED PNEUMONIA

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ABSTRACT

Mycoplasma pneumoniae (*M.pneumoniae*) is the leading atypical bacterial pathogen responsible for lower respiratory tract infection including infants, which is often associated with wheeze. We studied 55 infants up to 24 months of age having community acquired pneumonia presenting with acute wheeze. Cough and fever were most common symptoms while consolidation and infiltrates were most common radiographic abnormality present. *M. pneumoniae* was determined by detecting immunoglobulin (Ig) M antibody in blood by employing enzyme linked immunosorbent assay (ELISA) as well as detecting P1 adhesin gene by polymerase chain reaction (PCR) in nasopharyngeal aspirate. *M.pneumoniae* was found to be positive in 16.4% cases employing both the tests. Our study confirms the role of *M. pneumoniae* in infants presenting with wheeze which often misdiagnosed as viral pneumonia due to overlapping symptoms.

INTRODUCTION

Mycoplasma pneumoniae (*M. pneumoniae*) is an important bacterial agent responsible for primary atypical pneumonia of significant morbidity. It can cause both upper and lower respiratory tract infections. Rate of infection varies in community acquired bacterial pneumonia (CABP) from 4-8% endemically up to 20-40% during epidemic[1,2]. The proportion of infection varies according to age, most commonly infected are school age children and young adolescence. It is reported to have cause the infection even in neonates[3]. *M. pneumoniae* infection is usually underdiagnosed due to lack of or overlapping symptoms and no specific diagnostic criteria particularly in infancy as respiratory viruses are implicated as main causative agent in this age group. In 20–40% of children with acute *M. pneumoniae* respiratory infection are documented to have wheeze[4].

Detection of *M. pneumoniae* are being done by culture, serology and molecular methods. Slow growth and fastidious cultivation requirements make culture impractical. Presence of elevated IgM antibodies is diagnostic of recent infection in children > 6 months of age who are not likely to have experience repeated mycoplasmal infection as in adults[5]. Due to difficulty in obtaining convalescent sera, IgG detection in single acute specimen is non-specific due to presence of high background levels in some individuals[6]. Molecular tests have superior analytical and clinical sensitivity and shorter turnaround time, thus regarded as new gold standard[7]. *M. pneumoniae* could have epidemic potential in infants[8], leading to high mortality and extra pulmonary infections. The present study was undertaken to evaluate the clinical and radiological features of *M. pneumoniae* infection and its detection by employing IgM serology and polymerase chain reaction (PCR) in infants up to 24 months of age presenting with wheeze.

MATERIAL AND METHODS:

A total of 55 children aged 1month to 24 months presenting with wheeze, admitted in paediatrics department of Lok Nayak Hospital, New Delhi were included in this prospective, cross sectional study, from February 2014 to March 2015. The study was approved by the institutional ethics committee, Maulana Azad Medical College, New Delhi, India.

INCLUSION CRITERIA:

1. Age 1 month to 24 months ,
2. Children with acute wheeze.

EXCLUSION CRITERIA:

1. Any history of aspiration, immunodeficiency, tuberculosis, and congenital anomaly of respiratory tract.
2. Any hospital acquired pneumonia i.e. manifest after 48 hours of admission in hospital or 7 days of discharge from health care facility.

EVALUATION AND ENROLMENT OF PATIENTS:

Written informed consent was taken from the parents or legal guardians of children in accordance with standard bioethical norms. A detailed history and clinical examination were performed, and chest radiographs were obtained. Two ml blood sample was collected from all cases in a sterile centrifugation tube taking aseptic precautions. Serum separated and stored at -20°C. ELISA was performed for serum IgM antibodies against *M. pneumoniae* using commercially available ELISA based kits following the manufacturer's instructions (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany). The interpretative criteria were consistent with the recommendations of the manufacturer. Nasopharyngeal aspirates were collected employing a suction apparatus consisting of a disposable mucus sucker and sterile phosphate-buffered saline (pH 7.4). The samples were transported at 4°C to the laboratory and stored at -70°C till the detection of *M. pneumoniae* by PCR. DNA extraction was done and a 345 base pair region of P1 adhesin gene of *M. pneumoniae* was selected for amplification. Amplified PCR products were electrophoresed on an ethidium bromide stained agarose gel, along with a molecular weight ladder. A band of 345 base pairs was taken to be positive result.

STATISTICAL ANALYSIS

Data was recorded on a predesigned proforma and responses were coded for entry in the computer. All the entries were doubly checked for any possible keyboard errors. The difference of proportion between qualitative variables was tested using the Chi-square or Fischer's exact test. A p value less than 0.05 was considered as statistically significant.

RESULTS:

DEMOGRAPHIC PROFILE:

Total 55 infants included in study were divided into three groups of 1–6 months (Group 1), >6 months–12 months (Group 2), and >12 months to 24 months (Group 3). The number of M. pneumoniae in each of three group were equal. The differential presence of *M.pneumoniae* in wheezing

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infants across age groups was statistically insignificant. Total 19 female and 36 male were present in study. Percentage of male positive for *M. pnemoniae* were slightly higher than female but statistically insignificant [Table 1].

CLINICAL PROFILE:

Clinical profile of patients in the study population as coryza, cough, fever, and crepitations, in both *M. pnemoniae* positive and *M. pnemoniae* negative groups were comparable and there was not any statistically significant association between *M. pnemoniae* positive infection and clinical signs and symptoms [Table 2].

RADIOLOGICAL PROFILE:

In patients of the study population, the radiological features were extremely variable. The frequency of presence of infiltrates, consolidation, hyperinflation, pleural effusion, collapse of lung fields and normal radiological findings were comparable in both *M. pnemoniae* positive and *M. pnemoniae* negative groups and the difference between the two groups were statistically insignificant [Table 2].

MICROBIOLOGICAL PROFILE:

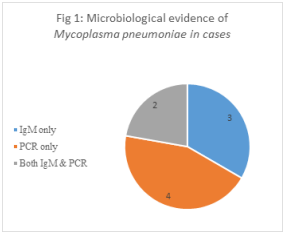
In the 55 patients of the study population, IgM antibody were detected in total 5 (9%) patients by ELISA, while PCR detected antigen in total 6 (10.9%) patients. Among which 2 patients, both antibody and antigen were detectable. Thus, total 9 (16.4%) cases of *M. pnemoniae* were positive employing both serology and PCR. [Fig1].

Table 1: Association of demographic profile of M. pnemoniae in children with wheeze

Character	M. pneumoniae positive; n=9; n(%)	M. pneumoniae negative; n=46; n(%)	Total; (n=55); n(%)	P -value
Age Group				
1-6 months	3(10.7)	25(89.2)	28(50.9)	0.29
>6 months-12 months	3(21.4)	11(78.6)	14(25.4)	0.67
>12 months - 24 months	3(23.1)	10(76.9)	13(23.6)	0.42
Sex				
Female	3(15.8)	16(84.2)	19(34.5)	1
Male	6(16.7)	30(83.4)	36(65.4)	

Table 2: Association of M.pneumoniae in patients with clinical and radiological findings

Character	M pnumoniae positive; (n==9), n (%)	M pnumoniae negative; (n=46), n (%)	Total (n=55); n (%)	P -value
Coryza	1(4.34)	22(95.6)	23(41.8)	0.06
Cough	8(18.2)	36(81.8)	44(80)	0.67
fever	7(15.9)	37(84.09)	44(80)	1
Crepitation	1(41.7)	23(95.8)	24(43.6)	0.06
Radiological findings				
Infiltrates	3(17.6)	14(82.3)	17(30.9)	1
Consolidation	3(27.3)	8(72.7)	11(20)	0.36
Hyperinflation	1(20)	4(80)	5(9)	1
Pleural Effusion	0(0)	1(100)	1(1.8)	1
Collapse of lung field	1(25)	3(75)	4(7.3)	0.52
Nothing abnormal detected	2(13.4)	15(88.2)	17(30.9)	0.7



DISCUSSION:

M. pneumoniae is a significant cause of CABP in children and can cause infections difficult to distinguish pneumonia caused by other respiratory pathogens. *M. pneumoniae* also affect infants and young children in epidemic periods[8]. Thus precise microbiological diagnosis are required by additional laboratory testing. Presently testing of acute phase sera for antibodies by qualitative tests continues to be commonly used for convenience. Enzyme immunoassay format has proven to be the most widely used and best studied[9].

In present study population positivity increases as age groups increases. 10.7% in < 6months, 21.4% in > 6 months to 12 months and highest 23.1% in >12 months to 24 months, which is in accordance with Huong Pie[10]et al who also reported highest rate of severe atypical community acquired pneumonia in children younger than 2 years of age. This is in contrast to study of Gadsby[8] et al who have reported highest number of cases came from those aged 1 year and younger. Our study has shown to have slight male preponderance of *M. pneomoniae* which corresponds to the study of Kashyap et al[11] and others[12,13]. This is in contrary to the study of Ke LQ[14] et al who reported a significant female association with *M. pneumoniae* infection.

The clinical manifestations of in *M. pneumoniae* are difficult to distinguish from other forms of viral or bacterial pneumonia due to significant overlap of signs and symptoms with no pathognomic finding. In our study also, no statistically significant correlation could be established between clinical characteristics and *M. pneumoniae* infection which is in accordance with previous studies that shown less specificity of clinical and laboratory features for predicting the microbial cause of community acquired lower respiratory tract infections in children and highlighted the need for laboratory confirmation[11,15]. However, in contrast Sun H[16] et al reported presence of fever with a maximum temperature of >39.0 °C for ≥3 days was significantly associated with 9 to < 12 month age group children. Radiological findings of *M. pneumoniae* infection are nonspecific and non-significant in our study similar to the study by Tharwat et al[17]. Consolidation (27.3%) was most common radiographic abnormality present in our study.

In present study *M. pneumoniae* was detected in 9 (16.4%) patients. This is in accordance to Kashyap *et al* (21.3%) and Prapphal et al (14.0%)[18]. Three patients were detected by IgM only while 4 patients detected by PCR only. In 2 patients both IgM and PCR were positive. Thus total 6 (10.9%) cases were detected by PCR and 5 (9%) cases were detected by IgM. High positivity of PCR detection in this study owing to its high analytical sensitivity[7] or could also be due to carrier state in some children. IgM negativity in PCR positive cases could be due to collection of sera before antibody rise as IgM rises 1 week after the onset of clinical disease. Similar IgM positivity of 9% were also reported Ramamoorthy et al[19] and PCR positivity of 10% was also shown in studies by Hadi et al[20] and Kumar et al[21]. Only 2 cases were detectable by both tests while 7 cases were having inconsistency between the PCR and serology in diagnosis of *M. pneumoniae* infection in our study. This inconsistency was also cited by Petitjean et al[22].

CONCLUSION:

Our data underline that *M. pneumoniae* plays a significant role in infants with wheeze in CABP in a low income country like India. As clinical and radiological features are insufficient to make diagnosis of this atypical agent, specific laboratory tests should be considered to enhance the early identification and timely management to reduce burden of hospitalization.

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