Original Article

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Paediatrics Section

Mycoplasma pneumoniae Infection and Subsequent Wheezing in Childhood Asthma- A Cohort Study

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ABSTRACT

Introduction: Prevalence of asthma in children <18 years is approximately 7-8%. *Mycoplasma pneumoniae* has also been found to be an important trigger of asthma. Many studies have shown the association of *M. pneumoniae* with acute exacerbation of asthma in adults. But the data is limited to determine the role of *M. pneumoniae* in causing subsequent wheezing in children with asthma.

Aim: To study the role of *M. pneumoniae* infection in causing subsequent wheezing episodes in asthma in children between 1 to 14 years of age.

Materials and Methods: This was a prospective cohort study conducted from October 2019 to October 2021 at the Paediatric Division of Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Consecutively 60 patients with bronchial asthma aged between 1 to 14 years were included during the first year of study period. The diagnosis of bronchial asthma was as per Global Initiative for Asthma (GINA) guidelines. Venous sample was tested for Immunoglobulin M (IgM) *M. pneumoniae* antibody with Calbiotech *M. pneumoniae* IgM Enzyme-linked Immunosorbent Assay (ELISA) kit. Clinical follow-up for subsequent wheezing was performed at 3, 6, and 12 months starting after 2 weeks of being declared symptom free. All data were recorded and analysed using Statistical Package for Social Sciences software (SPSS) version 22.0.

Results: Out of 60 patients, 46 (76.67%) were men and 14 (23.33%) were women with the mean age 5.56±4.21 years. Family history of wheezing was also recorded and found to be 38.33% among asthamatics (23/60). Among asthmatics, 23 cases (38.33%) were IgM positive. Episodes of subsequent wheeze were present in all the patients with an annual mean of 4.5±2.7 episodes. On subgroup analysis, mean wheezing episodes were documented as 5.43±3.00 in IgM positive group, which was statistically higher than IgM negative group in which mean wheezing episodes were 3.92±2.42 (p-value=0.036).

Conclusion: Subsequent wheezing was observed in all patients with asthma. The mean wheezing episodes were statistically more in *M. pneumoniae* infected asthmatics in comparison to non *M. pneumoniae* infected asthmatics.

Keywords: Children, Global initiative for asthma, Pulmonary infestation

INTRODUCTION

Mycoplasma pneumoniae is the smallest self-replicating bacteria. It can affect both the upper and lower respiratory tract resulting in a wide array of pulmonary and extra pulmonary manifestations [1]. According to a population-based study in the US, the prevalence of *M. pneumoniae* in Community Acquired Pneumonia (CAP) was, 16%, and 23% in <5, 5-9, and 10-17 years, respectively [2]. In India, the prevalence of *M. pneumoniae* infection in CAP ranges from 24 to 28% in various case series [3].

M. pneumoniae has recently been linked to the pathogenesis of asthma [4]. P1 protein is a surface adhesion molecule that plays an important role in the attachment of *M. pneumoniae* to the bronchial epithelial cells [5,6] and also acts as an allergen for certain individuals. Thus, *M. pneumoniae* plays a significant role in causing wheezing and in subsequent asthma [7,8]. There is a paucity of data regarding whether *M. pneumoniae* infection has a role in subsequent wheezing episodes in newly diagnosed childhood asthma [9,10]. Therefore, this study aimed to study the role of *M. pneumoniae* in causing subsequent wheezing episodes in asthma in children between 1 to 14 years of age.

MATERIALS AND METHODS

This was a prospective cohort study done at Paediatric Outpatient Department (OPD) and In-patient Department (IPD) of Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, India, between October 2019 to October 2021. The study was conducted after the approval from Institutional Ethics Committee. Based on previous 3 years patient data from our hospital, an estimated sample size of 80 newly diagnosed cases of asthma over one year was considered. Finally, 60 consecutive newly diagnosed cases of bronchial asthma aged 1 to 14 years formed the sample size.

Inclusion criteria: All consecutive children with newly diagnosed bronchial asthma aged between 1 to 14 years were screened for eligibility.

Exclusion criteria: Patients with co-morbidities such as heart diseases, other known respiratory problems, developmental delay, and any syndromes were excluded. Informed consent was obtained from the parents/caregivers of the patients.

Procedure

Diagnosis of bronchial asthma was made as per the 2019 Global Initiative of Asthma (GINA) guidelines [10]. The baseline demographic characteristics and clinical features were recorded in a proforma. Venous blood samples of 2 mL were taken on day one of OPD visit or hospitalisation. Then samples were centrifuged, and serum was separated. Calbiotech ELISA kit (A life Science Company) was used to test IgM against *M. pneumoniae*. When specific IgM ELISA titre was >100, the sample was considered positive for *M. pneumoniae*.

Relevant treatment like inhaled short acting beta agonists, inhaled steroids, mast cell stabilisers, and systemic steroids was administered as per GINA guidelines [10]. Follow-up was performed via interview either in person or telephonically at 3, 6, and 12 month intervals. Subsequent wheezing episodes were recorded through recall method from patient's attendants and/or evaluation of treatment

records. The number of wheezing episodes were recorded in both groups irrespective of the *M. pneumoniae* positivity status.

STATISTICAL ANALYSIS

All data were recorded and analysed by SPSS software. Obesity was considered at BMI ≥95th centile for age and sex whereas lean body weight was considered as BMI <5th centile for age and sex. Descriptive statistics were performed on clinical data of the study population and are expressed as mean±Standard Deviation (SD) for continuous variables and percentage for categorical variables. Categorical data was analysed using Chi-square test, for continuous variables with normal distribution. Independent sample t-tests and non parametric Mann-Whitney U tests were performed. A p-value of <0.05 was considered to indicate a statistically significant difference.

RESULTS

The study period included the first wave of COVID-19 pandemic, which resulted in a marginal drop in number of OPD/IPD patient visits. Out of 80, only 72 patients with asthma could be enrolled and out of 72, 12 patients were lost to follow-up. Thus, in the final analysis, 60 children with asthma were included.

As shown in [Table/Fig-1], the mean age was 5.56±4.21 years and majority of cases 35 (58.33%) were between 1 to 5 years age. Out of 60, males comprised of 46 (76.67%) cases. Obesity was present in 15% of asthmatics and 7% were found to be lean.

Demographic parameters	Value
Age (years)	
1-5	35 (58.33%)
6-10	14 (23.33%)
11-14	11 (18.33%)
Gender	
Male	46 (76.67%)
Female	14 (23%)
Family H/o wheeze	23 (38.33%)
Hospitalisation	14 (23.33%)
Weight (mean±SD) (kg)	17.85 ±10.72
Height (mean±SD) (cm)	100.82 ±26.57
Body mass index (mean±SD) (kg/m^2)	16.41±3.13

[Table/Fig-1]: Demographic parameters of patients with asthma.

Out of 60 patients, IgM against *M. pneumoniae* was found in 23 patients (38.33%). The demographic parameters were compared between subgroup of asthmatics (*M. pneumoniae* IgM positive and IgM negative) as shown in [Table/Fig-2].

Among 23 *M. pneumoniae* IgM positive cases and 37 IgM negative cases, mean age was 5.85±3.93 years and 5.38±4.42 years, respectively (p-value=0.678). The majority of patients were of the 1-5 years age group (52.17%) in IgM positive and 62% in IgM negative (χ^2=0.582, p-value=0.445). Mean BMI among IgM positive and IgM negative subgroups was observed as 15.72±2.36 kg/m^2 and 16.8±3.49 kg/m^2, respectively, which was statistically non significant. Family history of wheezing was also recorded and found to be statistically significant among IgM positive (14/23, 60%) and IgM negative subgroups (9/37, 24%). The number of hospitalisations were 17% among *M. pneumoniae* positive subgroup and 29% among *M. pneumoniae* negative subgroup, which was not statistically significant; χ^2=1.151, p-value=0.283.

Clinical parameters were compared in the *M. pneumoniae* positive and negative groups. As shown in [Table/Fig-3], fever was present in 91% of patients of the IgM positive group while in IgM negative, only 16% of patients had a fever, which was statistically significant (χ^2=32.310, p-value=0.0001).

Demographic parameters	MP IgM positive (n=23)	MP IgM negative (n=37)	Total	χ^2 value	p-value
Age					
1-5 years, n (%)	12 (52.17)	23 (62.16)	35	0.582	0.445
6-10 years, n (%)	7 (30.43)	7 (18.92)	14	1.051	0.305
>10 years, n (%)	4 (17.39)	7 (18.92)	11	0.022	0.881
Gender					
Male	20 (86.96)	26 (70.27)	46	2.207	0.137
Female	3 (13.04%)	11 (29.73)	14		
Family history of wheezing (n, %)	14 (60.87)	9 (24.32)	23	8.013	0.004
Hospitalisation (n, %)	4 (17%)	11 (29%)	15	1.151	0.283
Weight (mean±SD) (kg)	17.83±9.84	17.87±11.37	-	-	0.988
Height (mean±SD) (cm)	103.35±24.94	99.24±27.76	-	-	0.565
Body mass index (mean±SD) (kg/m^2)	15.72±2.36	16.83±3.49	-	-	0.183
Age (mean±SD), years	5.85±3.93	5.38±4.42	-	0.45	0.678
Total N (%)	23 (38.33)	37 (61.67)	60		

[Table/Fig-2]: Demographic variables among *Mycoplasma pneumoniae* IgM positive and negative cases.
MP: *M. pneumoniae*

Signs and symptoms	MP positive (n=23) n (%)	MP negative (n=37) n (%)	Total	χ^2 value	p-value
Fever	21 (91.30%)	6 (16.22%)	27	32.310	0.0001
Cough	18 (78.26%)	26 (70.27%)	44	0.463	0.496
Wheeze	21 (91.3%)	21 (56.76%)	42	8.061	0.004
Rhinorrhoea	14 (60.87%)	9 (24.32%)	23	8.013	0.004
Tachypnoea	9 (39.13%)	24 (64.86%)	33	3.795	0.051
Chest retractions n (%)	6 (26.09%)	10 (27.03%)	16	0.006	1
Cyanosis, n (%)	0	1 (2.70%)	1	0.632	0.426
Temperature (in Fahrenheit) (mean±SD)	100.11±0.57	98.62±0.88	-	-	0.0001
SpO_2% (mean±SD)	95.96±1.85	95.81±2.69	-	-	0.82

[Table/Fig-3]: Clinical features among IgM positive and IgM negative subgroups.
MP: *M. pneumoniae*

Wheeze was found in 91.3% in the IgM positive group and 56.76% in IgM negative group, which was statistically significant (χ^2=8.013, p-value=0.004). Rhinorrhoea was also found to be statistically significant; it was present in 60.87% in IgM positive group and 24.32% in IgM negative group (χ^2=8.013, p-value=0.004).

On the other side, cough (78.26% vs. 70.27%; p-value=0.496), chest retractions (26.09% vs. 27.03%; p-value=1) were present in both IgM positive and IgM negative subgroups; however, were not statistically significant.

One year follow-up of all cases after 2 weeks of being symptom free was performed in all patients to assess the number of new wheezing episodes. Episodes of subsequent wheeze were present in all the patients with a mean of 4.5±2.7 episodes in one year. On subgroup analysis, mean wheezing episodes were documented as 5.43±3.00 in IgM positive subgroup which was statistically higher than IgM negative group in which mean wheezing episodes were 3.92±2.42 (p-value=0.036) [Table/Fig-4].

Wheezing episodes	IgM positive (n=23)	IgM negative (n=37)	p-value (Mann-Whitney U test)
First 3 months (Mean±SD)	1.42±1.04	0.86±0.38	0.026
4 to 6 months (Mean±SD)	1.89±0.99	1.65±0.74	0.071
7 to 12 months (Mean±SD)	2.92±1.06	1.49±0.53	0.034
Total 1 year (Mean±SD)	5.43±3.00	3.92±2.42	0.036
Overall mean wheezing episodes (Mean±SD)	4.5±2.7		

[Table/Fig-4]: Mean of subsequent wheezing episodes among asthmatics.

It was observed that there was no significant difference between the mean wheezing episodes among in-patients and out-patients with the IgM+ asthma group (p-value=0.818) [Table/Fig-5].

Mean wheezing episodes	Inpatient (14)	Outpatient (46)	p-value (Mann-Whitney U test)
Asthma (n=60)	4.07±1.89	4.63±2.94	0.505
IgM positive Asthma (n=23)	5.75±1.25	5.36±3.26	0.818
IgM negative Asthma (n=37)	3.40±1.62	4.10±2.63	0.455

[Table/Fig-5]: Mean of subsequent wheezing episodes among inpatient and outpatient asthmatics.

DISCUSSION

This study analysed the role of *M. pneumoniae* infection in newly diagnosed asthma and its impact in causing subsequent wheezing episodes in children. The mean age was 5.56±4.21 years and majority (58.33%) of cases were between 1 and 5 years age. Similar to our study, Meza ED et al., [11] and Kassisse E et al., [12] conducted an observational study on asthmatics to analyse its relation with *M. pneumoniae*, they found the mean age 6.3±3.6 years and 5.0+2.01 years, respectively, and was found to be more prevalent in <5 years of age group; however, was not statistically significant.

M. pneumonia infection has been associated with subsequent wheezing episodes and asthma in current study. Gao S et al., [13] conducted a prospective study in a China hospital from 2012 to 2014. Among 78 cases with asthma in the acute phase, *M. pneumoniae* infection was found in 38 cases (48.71%), and among 71 cases with asthma in the convalescent phase, *M. pneumoniae* infection was found in 22 cases (30.98%). A paediatric study suggested that *M. pneumoniae* infection plays an important role in the asthma exacerbations [12]. A cross-sectional study was conducted by Meza ED et al., [11] to determine the prevalence of *M. pneumoniae* infection in children aged 2-15 years with acute asthma exacerbation from 2010 to 2012 in Colombia. A total of 169 children with asthma exacerbation were enrolled. Polymerase Chain Reaction (PCR) was performed on nasopharyngeal aspirates and *M. pneumoniae* was found to be prevalent in 12.4% of cases.

A prospective cross-sectional study was conducted by Iramain R et al., [14] from 2007 to 2013 at a hospital in Paraguay to determine the prevalence of *M. pneumoniae* and its relationship to severe asthma. Total 82 children aged 2-18 years were enrolled. IgM detection for *M. pneumoniae* was performed on serum samples and was found in 22.2% of cases with severe asthma, 6.9% of cases with stable asthma, and was not found in the control group. Thus, *M. pneumoniae* plays a role in the development of severe asthma. A prospective case-control study was carried out by Kassisse E et al., [12] at a hospital in Venezuela, from 2015 to 2016, to determine the prevalence of *M. pneumoniae* infection in patients aged 2-12 years with acute asthma exacerbation. *M. pneumoniae* IgM was detected in 60 out of 130 patients of asthma (46.15%). This suggests a relation of *M. pneumoniae* with severe acute asthma exacerbation.

In a study from India, Kumar S et al., [15] conducted a prospective cohort study at Maulana Azad Medical College, New Delhi, to assess the clinical association between exacerbation of asthma and *M. pneumoniae* infection in children. Serum samples were analysed using ELISA for the detection of IgM antibodies to *M. pneumoniae* and were found to be positive in 21 patients (42%) in known asthmatics presenting to emergency and 4 patients (13.3%) with controlled asthma. Thus, there was an association of *M. pneumoniae* infection with asthma exacerbation. Similarly, present study found that the prevalence of *M. pneumoniae* among asthmatics was 38.33% (23 out of 60 patients) by using the IgM ELISA method.

Data regarding the role of *M. pneumoniae* infection in future course of asthma in children is scarce. A study from Korea by Rhim JW et al., [16] found that 6.2% of patients (31/501) aged between 0 to 15 years had experienced subsequent wheezing episodes after initial infection with *M. pneumoniae* over 3 years of follow-up. A retrospective analysis was performed by Kong K et al., [9] to identify the possible risk factors associated with wheezing among children (aged 28 days to 18 years) diagnosed with *M. pneumoniae* infection at a hospital in Shanghai from 2019 to 2020. Among 1,181 patients with *M. pneumoniae* infection, 295 (25.0%) suffered from wheezing. According to current observational data, future episodes of subsequent wheeze occurred in all asthmatics over 1 year of follow-up. Overall mean wheezing episodes in asthmatics were 4.5±2.7 episodes per year. On subgroup analysis, mean wheezing episodes were more in the IgM positive group (5.43±3.00) in contrast to IgM negative group (3.92±2.42) and it was statistically significant.

Since *M. pneumoniae* infection may also be a trigger for asthma, it was essential to establish its role in subsequent wheezing in asthma. This study is unique because it is a prospective observational study to analyse the role of *M. pneumoniae* infection among asthmatic children in future exacerbations of asthma. Further research should be conducted involving a larger cohort of children to analyse the role of *M. pneumoniae* in subsequent wheezing episodes.

Limitation(s)

Only IgM antibody detection for *M. pneumoniae* was done for the diagnosis. The present sample size of the study was less than anticipated and was probably due to the drop in OPD and IPD visits because of the prevailing COVID-19 pandemic.

CONCLUSION(S)

M. pneumoniae infection is an important trigger for asthma. This infections also play an important role in the future exacerbation of asthma and the number of exacerbations has been found to be statistically more in the infected group as compared to non infected group amongst asthmatics. Prevalence of *M. pneumoniae* infection in asthmatics has been found to be 38.3%. Therefore, efforts must be made to screen all patient of asthma for *M. pneumoniae* infection and treat accordingly. Further large scale, multicentre studies are required to explore this relationship.

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