Research Article

Seroprevalence of Mycoplasma Pneumoniae in Children with Pneumonia

Ujala Ilyas[1], Jalees Khalid Khan[2], Muhammad Faheem Afzal[3], Hina Bukhari[4], Saeed Ahmad[5], Muhammad Haroon Hamid[6]

[1,2,4,5] Department of Pathology, King Edward Medical University, Lahore; [3,6] Department of Pediatrics, KEMU/ Mayo Hospital, Lahore

Abstract

Objective: To determine the seroprevalence of Mycoplasma pneumoniae in children clinically diagnosed as pneumonia

Methods: This descriptive study was conducted with the collaboration of Pathology and Pediatrics Departments from November 2018 - April 2019 in King Edward Medical University, Lahore. Total 75 children aged ≤ 12 years of either gender, clinically diagnosed as pneumonia were included by non-probability, purposive sampling. After diagnosis, 2 ml serum was obtained from venous blood samples and was stored in yellow vacutainers at (-20~ -70) °C till assayed. Acute & convalescent phase serum specimens were taken. The specific IgA antibodies against Mycoplasma pneumoniae were detected by ELISA. SPSS version 21 was used for data entry and analysis. Quantitative variables such as age were showed as mean ± SD. Qualitative variables such as gender were showed as frequency & percentages. Chi- Square test was applied to see the association between between age and seroprevalence. p-value <0.05 was taken as significant.

Results: Total 20 children (26%) were sero-positive against Mycoplasma pneumoniae. In acute phase, 12(16%) patients and in convalescent phase 16(21.3%) patients had IgA antibodies in serum against Mycoplasma pneumoniae infection.

Conclusion: Seroprevalence of Mycoplasma pneumoniae was 26.7% in children clinically diagnosed as pneumonia determined by IgA antibodies in serum.

Corresponding Author | **Dr. Ujala Ilyas,** Department of Pathology, King Edward Medical University, Lahore **Email:** ujalailyas.ui@gmail.com

Key Words: Pneumonia, Children, Mycoplasma pneumoniae, IgA antibodies.

Introduction:

Pneumonia is the one of the largest infectious cause of mortality in children worldwide, accounting for 15% of all deaths of children under five years.[1] Pneumonia is caused by a range of micro-organisms. Streptococcus pneumonia is the predominant bacterial cause of typical pneumonia. However, one-fifth of community-acquired pneumonia cases are atypical. Mycoplasma pneumoniae, is the commonest causative agent of primary atypical community acquired pneumonia, it was first identified by Eaton et al.[2] An epidemiological analysis of mycoplasma pneumoniae conducted on spainsh children from 2010-2015 showed that prevalence of mycoplasma pneumoniae was 26.5%.[3] The signs and symptoms of this infection may be similar to those of influenza. However, the laboratory diagnosis of M. pneumoniae has limitations, as the traditional method for culture of the organism requires fastidious and exacting growth conditions, paired sera are needed for sero-diagnosis and the high cost of molecular diagnostic methods can be problematic in resource-limited countries.[4] The cold agglutinin test was used

in the past to diagnose M. pneumoniae infection as a low-cost approach, but its very low sensitivity and specificity compromised its utility.[5] Serological methods (IgA, M, G by ELISA) may be used for diagnosis as cost effective tool to focus on appropriate antibiotic prescription and thereby minimize the development of antibiotic resistance.[6] 134 out of 746 children in Denmark were tested positive for M. pneumoniae by PCR or serology.[7] In our region, M. pneumoniae infections are not widely diagnosed or confirmed by laboratory tests and the suspected cases are treated empirically. Empirical therapy should ideally be guided by local prevalence data, including the spectrum of organisms involved and their antibiotic resistance profiles, as well as clinical and laboratory criteria. However, local prevalence data are currently not available for this infection. Having such data would refine the empirical antibiotic guidelines, minimize inappropriate use of broad-spectrum antibiotics and facilitate patient management. Therefore, the rationale of this study was to find out the seroprevalence of *Mycoplasma pneumoniae* in children clinically diagnosed as pneumonia.

Methods:

This study was conducted with the collaboration of Pathology and Pediatrics Departments from November 2018 – April 2019 in King Edward Medical University, Lahore. Total 75 children ((estimated by using 95% confidence level, 8% absolute precision with expected percentage of pneumonia as 14.5%[8]) aged ≤ 12 years of either gender, clinically diagnosed as pneumonia (Fever, cough, difficulty in breathing, and/or age appropriate tachypnea[9]) were included by non- probability, purposive sampling. Children with proven immunodeficiency / immunosuppression, children with history of autoimmune disorders, recent use of systemic corticosteroids (less than 30 days), having taken anti-Mycoplasma (macrolides) treatment & history of recent use of immunoglobulins were excluded from the study.

The study was approved by the institutional review board and was funded by research grant of King Edward Medical University, Lahore. Written informed consent was obtained from study participants and/or their parents. Demographic details (name, age, gender) were also noted. Samples were collected from Pediatrics department and were processed at Pathology department of KEMU Lahore. Children were enrolled as per operational definition (Fever, cough, difficulty in breathing, and/or age appropriate tachypnea.[9] After diagnosis, 2 ml serum was obtained from venous blood samples and was stored in yellow vacutainers at (-70)°C till assayed. Acute phase serum samples were obtained after enrollment/ admission whereas convalescent serum specimens were taken 2-3 weeks later to provide reliable results. Children or caregivers were asked to follow up for convalescent serum samples. The specific IgA antibodies against mycoplasma pneumoniae were determined in serum samples of patients using a commercial ELISA kit (NOVA TEC ELISA IgA Kit) according to manufacturer's instructions. The antigens used in the sero mycoplasma pneumoniae kit are the P_1 membrane proteins, which was major immunogen of mycoplasma pneumoniae. A specimen was considered positive for IgA when the absorbance value was equal to or greater than that of the cutoff serum specimen included in the kit. Results were evaluated by reading absorbance of wells by ELISA reader spectrophotometrically at wavelength of 450 nm. The intensity of coloration is directly proportional to amount of mycoplasma pneumoniae antibodies present in the test sample.

SPSS version 21 was used for data entry and analysis. Quantitative variables such as age were showed as mean ± SD. Qualitative variables such as gender were showed as frequency & percentages. Chi- Square test was used to see the association between age and seroprevalence. p-value ≤ 0.05 was taken as significant.

Results:

Mean age of patients in this study was 3.85 ± 3.09 years. Among patients 48(64%) were males and 27 (36%) were females. Out of 75, 15 children were less than 1 year of age, 22 children were between 1 to 3 years, 31 were between 4 to 9 years and 7 were between 10-12 years of age. (Figure I)

Total 20 patients were seropositive for IgA mycoplasma pneumoniae, 12 cases were positive in acute phase, 16 cases were positive in convalescent phase Table (I). Only 4 cases were positive, only in acute phase but negative in convalescent phase, follow up of those patients was late (after the recommended time i.e. 2-3 weeks). It showed that IgA levels declined rapidly that's why these children (who presented late for 2nd serum sample) were negative in convalescent serum sample. This shows that IgA

antibodies are good diagnostic indicator for current infection. IgA Elisa findings showed that in acute phase 12 (16%) patients and in convalescent phase 16 (21.3%) patients were positive for Mycoplasma pneumoniae Table (I). IgA ELISA is also helpful in diagnosing mycoplasma pneumoniae infection in early stage (acute phase). A child who was positive in both acute and convalescent phase that child was counted as one patient, because few children were positive both in acute and convalescent phase, therefore total number of positive patients were 20 (Table I). Mycoplasma pneumoniae infection was more prevalent in 4 to 9 years of age group patients (22.6% in acute phase & 29% in convalescent phase). No significant association was seen between Elisa IgA results (acute & convalescent phase) in relation to age of patients. i.e. p-value (acute phase) = 0.336 & p-value (convalescent phase) = 0.309 (Table II) Out of 75 children, 36 children (48%) were going to school and 39 (52%) were not going to school. Among those children who were going to school, 12 children (33.33%) were found sero-positive whereas 8 children (20.51%) were sero-positive among those who were not going to school. (pie chart I)

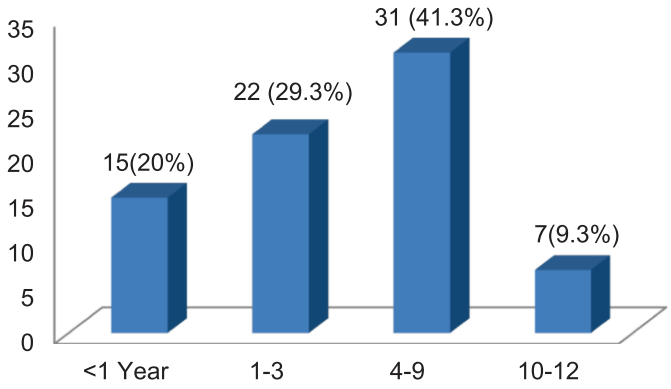

Figure 1: *Age Distribution of children*

Table I: *ELISA findings of IgA Mycoplasma pneumoniae in acute & convalescent phase (n = 75)*

	Acute Phase	Convalescent phase
Positive	12 (16%)	16 (21.3%)
Negative	63 (84%)	59 (78.7%)
Total	**75**	**75**

Table II: *ELISA Findings for Mycoplasma pneumoniae in acute convalescent phase in relation to age of patients (n = 75)*

	Acute Phase			Convalescent phase		
	Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**
< 1 years	1 (6.7%)	14 (93.3%)	**15**	2 (13.3%)	13 (86.7%)	**15**
1-3	4 (18.2%)	18 (81.8%)	**22**	5 (22.7%)	17 (77.3%)	**22**
4-9	7 (22.6%)	24 (77.4%)	**31**	9 (29%)	22 (71%)	**31**
10-12	0 (0%)	7 (100%)	**7**	0 (0%)	7 (100%)	**7**
Total	**12**	**63**	**75**	**16**	**59**	**75**
p-value		**0.336**			**0.309**	

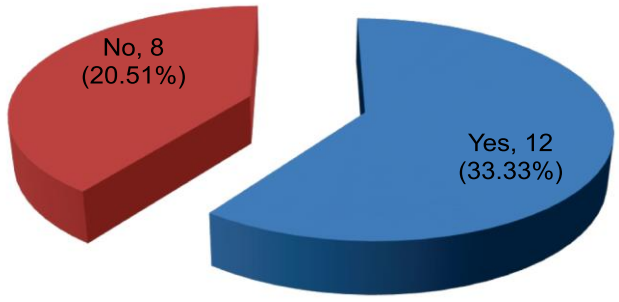

Figure 2: *Mycoplasma Pneumoniae infection with history of schooling*

Discussion:

In this study, Mycoplasma pneumoniae was diagnosed with the help of IgA antibodies by ELISA. Frequency of Mycoplasma pneumoniae was 26% in children. A study in Argentina showed prevalence of anti-mycoplasma pneumoniae antibodies was 14.6% in 0-12 years old children.[10] Likewise, Shenoy et al. reported that 24% pneumonia cases in the hospitalized children are caused by M. pneumoniae.[11] A study conducted during 2011-2012 in England using

PCR on swabs of nasopharynx and oropharynx showed that incidence of infection was 9% in children less than 16 years of age group whereas 14.3% incidence in 5 to14 years old children. According to laboratories' serological data, M.P infections were more common in 5 to 9 years old children (18%) as compared to 0 to 4 years old children (10.4%).[12] It corresponds to our findings in which Mycoplasma pneumoniae infection was more prevalent in 4 to 9 years of age group patients. Similar study in Den-mark showed the highest rate of M. pneumoniae positive samples was in school aged children (65%), preschool (30%), and less than 2 years (4%).[7]

Although many serological tests are available for mycoplasma pneumoniae infection but ELISA is the most specific and sensitive test. Studies of kumar et al. and kashyap et al. reported 34% and 21.3% seropositivity of M.P infection in children with community acquired respiratory tract infections respecttively.[13] At Aga Khan University Hospital, Karachi, a study conducted on 124 adult patients who were diagnosed with community-acquired pneumonia. Among the 124 patients the most common etiologic agent identified was Mycoplasma pneumoniae (n = 21, 17%), followed by Chlamydia pneumoniae (n = 15, 12%), Streptococcus pneumoniae (n = 9, 7%), Haemophilus influenzae (n = 2, 1.6%), Klebsiella pneumoniae (n = 2, 1.6%) and Staphylococcus aureus (n = 1, 0.8%).[14] In the past years, only a few researches have reported the significance of Mycoplasma pneumoniae specific IgA antibodies in diagnosing Mycoplasma pneumoniae infections Yan Dong studied that infection initially, mycoplasma pneumoniae specific IgM and IgA positive cases were 63.6% and 33.8%, respectively. After 1 week of infection, IgM and IgA positive cases in Mycoplasma pneumoniae infection were raised to 97.5% and 56.3%, respecttively.[15] Massimo De Paschale found in his study that diagnosis of mycoplasma pneumoniae by detection of IgA increased twice the diagnostic yield in old patients as compared to young age patients when he used IgA along with IgM, in place of using only IgM.[16] Wei-Ju Lee [17] suggested that detection of IgA is lesser sensitive than IgM in diagnosing pneumonia caused by mycoplasma pneumoniae in children of school age and adults because he found more positive results of mycoplasma pneumoniae by using IgM specific antibodies. Lih-Ju Lin[18] found in the study that when IgA and IgM antibodies are detected simultaneously for the detection of MP infection, sensitivity raised to 71.4% as compared to when either IgA or IgM tested alone positive results were less than 50% for each antibody. Hence, he con-cluded that detecting IgA and IgM antibodies simultaneously increase the sensitivity for diagnosis of mycoplasma pneumoniae infection.

This study has few limitations. We did not go for IgM and IgG analysis for better comparison with IgA results. We did not had any gold standard test for diagnosis of Mycoplasma pneumoniae to compare with. Results from smaller sample size may not be generalized. Larger multicenter studies are needed to validate these results.

Conclusion:

Seroprevalence of Mycoplasma pneumoniae was 26.7% in children clinically diagnosed as pneumonia determined by IgA antibodies in serum.

Ethical Approval: Given
Conflict of Interest: The authors declare no conflict of interest
Funding Source: Research Grant of King Edward Medical University, Lahore.

References:

1. World Health Organization. Pneumonia. [online] 2019 [cited 2020 July 27]. Available from: https://www.who.int/news-room/fact-sheets/detail/pneumonia.
2. Saraya T. The history of Mycoplasma pneumoniae pneumonia. Front Microbiol. 2016;7(2):364.
3. Aguilera-Alonso D, Ruiz RL, Rubiano JC, García MM, García IV, Mochón MD, et al. Epidemiological and clinical analysis of community-acquired mycoplasma pneumonia in children from a spanish population, 2010-2015. Anales de Pediatría (English Edition). 2019;91(1):21-9.
4. Loo LH, Soong HY, Maiwald M, Tee NW. Assessment of Genotypic Macrolide Resistance among Mycoplasma pneumoniae Infections in Children in Singapore. Ann Acad Med Singapore. 2017;46 (7) :290.
5. Wijesooriya WRPLI, Sunil-Chandra NP, Perera J. Reliability of cold agglutinin test (CAT) for the detection of patients with Mycoplasma pneumoniae pneumonia. SLJID. 2016;28(4):6.

6. Loens K, Ieven M. Mycoplasma pneumoniae: current knowledge on nucleic acid amplification techniques and serological diagnostics. Front Microbiol. 2016;7(2):448.
7. Søndergaard MJ, Friis MB, Hansen DS, Jørgensen IM. Clinical manifestations in infants and children with Mycoplasma pneumoniae infection. PLoS One. 2018;13(4):e0195288.
8. Medjo B, Atanaskovic-Markovic M, Radic S, Nikolic D, Lukac M, Djukic S. Mycoplasma pneumoniae as a causative agent of community-acquired pneumonia in children: clinical features and laboratory diagnosis. Ital J Pediatr. 2014;40(1):104.
9. Fitzner J, Qasmieh S, Mounts AW, Alexander B, Besselaar T, Briand S, et al. Revision of clinical case definitions: influenza-like illness and severe acute respiratory infection. Bulletin of the World Health Organization. 2018;96(8):122-8.
10. Fenelli GS, Chiolo MJ, Torres FA, Balbaryski J, Domínguez P, Ossorio MF, et al. Seroprevalence of anti-Mycoplasma pneumoniae antibodies in otherwise healthy children. Rev Chil Pediatr. 2020; 91(3):347-52.
11. Kumar S. Mycoplasma pneumoniae: A significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections. Indian J. Med. Res. 2018;147(1):23.
12. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. Mycoplasma pneumoniae from the respiratory tract and beyond. Clin. Microbiol. Rev. 2017;30 (3):747-809.
13. Kumar S, Garg IB, Sethi GR, Kumar S, Saigal SR. Detection of immunoglobulin M and immune globulin G antibodies to Mycoplasma pneumoniae in children with community-acquired lower respiratory tract infections. Indian J. Pathol. Microbiol. 2018;61(2):214.
14. Zubairi AB, Zafar A, Salahuddin N, Haque AS, Waheed S, Khan J. Atypical pathogens causing community-acquired pneumonia in adults. JPMA. The Journal of the Pakistan Medical Association. 2012;62(7):653.
15. Dong Y, Lv W, Lin Z. [Retracted] Value of serum Mycoplasma pneumoniae immunoglobulin in the diagnosis of mycoplasma-related pneumonia in newborns. Exp Ther Med. 2018;16(2):1036-40.
16. De Paschale M, Cerulli T, Cagnin D, Paganini A, Manco MT, Belvisi L, et al. Can the detection of IgA anti-Mycoplasma pneumoniae added to IgM increase diagnostic accuracy in patients with infections of the lower respiratory airways? World J Clin Infect Dis. 2016;6(4):67-72.
17. Lee W-J, Huang E-Y, Tsai C-M, Kuo K-C, Huang Y-C, Hsieh K-S, 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;24(1):e00471-16.
18. Lin L-J, Chang F-C, Chi H, Jim W-T, Huang DT-N, Kung Y-H, et al. The diagnostic value of serological studies in pediatric patients with acute Mycoplasma pneumoniae infection. J Microbiol Immunol Infect. 2020;53(2):351-6.