Isolation of *Mycoplasma pneumoniae* in Bronchial Asthma Patients

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ABSTRACT

BACKGROUND
Asthma is a chronic inflammatory disorder which has many triggering factors. *Mycoplasma pneumoniae* has been found to be a cause of exacerbations and chronicity of asthma. The present study is undertaken to determine the prevalence of *Mycoplasma pneumoniae* in lower respiratory tract of adults with asthma by isolating the organism by sputum culture.

METHODS
Hundred patients of asthma were studied for *Mycoplasma* by sputum culture. Baseline investigations and assessment of severity of asthma by symptom score was done.

RESULTS
18% of patients had *Mycoplasma* positive in sputum culture. There was statistically significant difference between the *Mycoplasma* positive and negative groups when the daytime symptoms were analysed.

CONCLUSIONS
Mycoplasma infection was associated with asthma patients. Parameters like daytime symptom score and eosinophil count showed statistically significant difference between the *Mycoplasma* positive and negative group, whereas age, sex, family history of asthma, X Ray PNS, Gram stain and Ig E levels did not show any statistically significant difference between the two groups.

KEYWORDS
Asthma, *Mycoplasma pneumoniae*, Sputum Culture, Daytime Symptom Score, Pyogenic Culture

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BACKGROUND

Asthma is a chronic inflammatory disorder of the airways mediated by many cells, particularly mast cells, eosinophils and T lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night or early morning. The inflammation causes airway hyper responsiveness to a variety of stimuli[1] like allergens, pharmacological stimuli, environmental and air pollutants, occupational factors, infections, exercise and emotional factors.[2]

Among the triggering factors, respiratory infections mainly by viruses plays an important role, followed by bacteria including atypical organisms like *Mycoplasma* and chlamydia.[3] Different studies have shown an association between *Mycoplasma* pneumonia and asthma, acting as a cause of acute exacerbations[4] and also as a cause of chronicity of asthma.[5] A few studies also postulated that *Mycoplasma* can also act as initiator of asthma. Different pathophysiologic mechanisms are also put forward for this association between *Mycoplasma* and asthma. Most of the studies[4,5,6,7] have shown an association by isolating the organism by serologic methods, throat swab culture and also by sputum analysis.[8] The present study is undertaken to determine the prevalence of *Mycoplasma pneumoniae* in lower respiratory tract of adults with asthma by isolating the organism by sputum culture.

We wanted to determine the incidence of *Mycoplasma pneumoniae* in bronchial asthma patients by isolation of the organism by sputum culture.

METHODS

This study was conducted in the Department of Chest Medicine in association with Microbiology department in a tertiary care hospital in South India. Ethical permission was obtained from the institutional review board and the period of study was 2 years. Participants represented various parts of South India and were enrolled with consecutive sampling technique. Eligible participants were approached, and Informed consent was obtained before enrolling in the study. Hundred consecutive patients of bronchial asthma were studied.

Inclusion Criteria

Proven cases of bronchial asthma on the basis of clinical symptoms, signs, pulmonary function tests, irrespective of their age were taken.

Exclusion Criteria

- Patients on antibiotics within the last 48 hours.
- Pregnant women with asthma.
- COPD.
- Cardiac asthma.
- Patients who were not able to produce satisfactory sputum even after sputum induction methods.
- Patients requiring ICU care

All patients were subjected to baseline investigations like haemoglobin, total count, differential count (to get eosinophil count), sputum for gram stain, pyogenic culture and sensitivity, ECG, X ray PNS, X ray chest and total IgE levels. Patients were clinically assessed for severity of asthma by the asthma symptom score devised by Black et al. Sputum was sent for *Mycoplasma* culture after administration of first dose of antibiotic immediately to microbiology laboratory.

Sputum Collection

Adequate amounts of deeply coughed out sputum, preferably early morning was taken in a sterile plastic container with a lid and with precautions to prevent contamination with saliva. PPLO broth was used as transport medium. Hypertonic saline nebulisation was given to patients who were not able to give adequate sputum and samples were sent immediately for *Mycoplasma* culture and results were obtained after 4 weeks. Sputum samples were inoculated into *Mycoplasma* broth and *Mycoplasma* isolation agar media, sealed and incubated for 2-4 weeks in a microaerophilic atmosphere. The plates were observed under light microscopy for fried egg colony. Suspected *Mycoplasma* colonies were subcultured to reduce bacterial overgrowth and to increase yield.

The study extended for a period of two years and participants were enrolled as required for the study. Data was entered into excel and doubled checked for eliminating the data entry errors. To maintain the confidentiality, data was entered anonymously using separate codes and personal identifications were avoided.

RESULTS

One hundred asthma patients were studied of which 52 were males and 48 females. 18 patients sputum grew *Mycoplasma* and 82 were negative for *Mycoplasma*. The mean age of the study group was 36 years which showed no difference between the *Mycoplasma* positive and negative groups. The sex distribution of the sputum *Mycoplasma* positive group showed that 6 were males and 12 were females. On analysing the study group with respect to family history of atopy and asthma, there was no statistically significant differences between the culture positive and negative groups. The mean duration of asthma in the group was studied. The longest duration was 40 years and shortest duration was 2 months. There was no statistical difference between the *Mycoplasma* positive and negative groups. The patients in the study group were divided into 5 classes according to the day time symptoms as devised by Black et al. It was found that there was statistically significant difference between the *Mycoplasma* positive and negative groups when the day time symptoms were analysed. The exacerbation inside the study group when compared with

Mycoplasma positive and negative groups showed no statistical difference. When eosinophil count was studied there was statistically significant difference between the *Mycoplasma* positive and negative patients. Analysis of Immunoglobulin E levels showed that *Mycoplasma* positive group had high levels, but it was not significant. Study of dynamic obstruction of airways measured in terms of FEV1/ FVC in the group showed no statistical difference between *Mycoplasma* positive and negative patients. The number of patients with exacerbation of asthma at screening was found to be 18. Gram staining for respiratory pathogens in the study group showed no statistical significance. The study compared the x-ray features of sinusitis with respect to *Mycoplasma* positive and negative patients and there was no statistical difference between them.

Variable		*Mycoplasma* Positive	*Mycoplasma* Negative	p
Number		18	82	
Mean Age in years ±		36.11±17.01	36.13±18.48	0.996
Sex	Males	6	46	0.80
	Females	12	36	
Family History of Asthma	Positive	4	28	0.326
	Negative	14	54	
Mean duration of asthma ± SD	In Months	77.78±72.06	115.61±97.66	0.240
Exacerbation of asthma	Present	3	8	0.011
	Absent	15	74	
Mean eosinophil count ± SD		5.83±2.79	6.89±4.64	0.018
Mean IgE ± SD		1335.82±872	1019±994.7	0.138
Mean FEV1/FVC±SD		85±233	83±445	0.408
Sinusitis in X-ray PNS	Present	7	40	0.446
	Absent	11	42	

Table 1. Comparing the Different Variables in Mycoplasma-Positive and -Negative Patients

Variable		*Mycoplasma+*	*Mycoplasma-*	p Value
Symptom Score	0	0	0	0.01
	1	1	0	
	2	1	17	
	3	6	54	
	4	10	11	
	5	0	0	
Gram stain	0	12	40	0.518
	1	2	19	
	2	0	1	
	3	4	22	

Table 2. Gram Stain Results in Mycoplasma Positive and Negative Patients

Gram stain: 0-no organism, 1- strep pneumonia, 2- h influenza, 3-strep pneumonia & H influenza

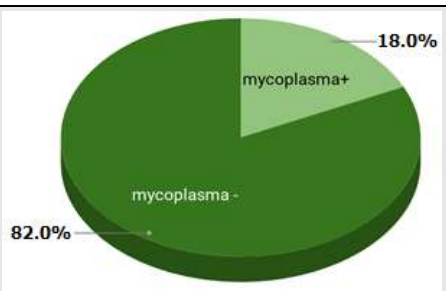

Figure 1. Incidence of Mycoplasma

DISCUSSION

Out of the hundred asthmatics studied, eighteen patients sputum grew *Mycoplasma*. Average age of the patients in study group was in the 4th decade. This is because this was a tertiary care hospital and paediatric patients were not included. Gil et al[9] studied the isolation of *Mycoplasma* in asthmatics and there was no increased incidence in any particular age group, like this study. Hjordis[10] showed that *Mycoplasma* infections were highest in school children and second highest in children <5 years of age and third in women in 30s, usually caretakers of school children. But this does not apply to our study, in which we tried to detect *Mycoplasma* in asthmatics. Sex distribution among *Mycoplasma* positive and negative patients, showed that most of the *Mycoplasma* positive patients were females (12/18) 66.3%, compared to males, 33.7%, but this was statistically insignificant. These findings were comparable to Gills[9] study of *Mycoplasma* infection in asthmatics. The duration of asthma among the *Mycoplasma* positive and negative group did not show statistical difference, which is at par with Gils[9] study.

Asthma symptom score, for showing severity of asthma were higher in *Mycoplasma* positive patients compared to negative patients. Previous studies by Sabeto et al,[11] Seggev et al,[12] and Freymouth et al[13] showed that *Mycoplasma* can cause exacerbation of asthma and make asthma control difficult, but treatment with macrolide antibiotics for 6 weeks showed a significant improvement in symptoms and pulmonary function tests when compared to placebo as shown by Martin et al.[14] In our study, only 3 out of 18 (16.66%) patients were having acute exacerbation, but *Mycoplasma* as the causative organism in the above cases cannot be definitely told since these patients improved without specific anti *Mycoplasma* antibiotics. Espisito et al[15] and Seggev ET al[12] showed exacerbation of asthma in about 21% *Mycoplasma* infections. Lieberman et al[16] found 18% exacerbation with *Mycoplasma pneumoniae* in asthmatics. Our study also showed similar incidence of exacerbation by *Mycoplasma* even though there was no statistical significance.

When compared *Mycoplasma* positive and negative patients eosinophils were decreased in patients with *Mycoplasma*. Previous study by Shimizu ET al[17] showed that on follow up of patients with *Mycoplasma*, eosinophil count gradually increased from acute infection to convalescent phase. In our study we didn't follow up eosinophil count during convalescent phase. Immunoglobulin E levels were more in *Mycoplasma* positive patients in our study, but this was not statistically significant. Study by Tipirineni et al[18] and Nicholson et al[19] showed that total Ig E levels were increased in patients with *Mycoplasma* as indicated by serologic methods. Shimizu et al[17] also showed specific Ig E antibody to *Mycoplasma* which was elevated during acute infection and decreased during the convalescent period and postulated to have a pathogenic role in exacerbation of asthma. In yet another study, Kraft et al[6] suggested that Ig E levels were not raised in a group of stable asthmatics and postulated that absence of the antibody response will keep the organisms in the lower airway without eradication of the organism.

When pulmonary function tests were compared with *Mycoplasma* positive and negative patients, our study did

not show much difference. Previous studies by Wongtin[20] and Jacqueline[21] showed that usual parameters of pulmonary function tests could be deranged transiently or permanently in patients with *Mycoplasma* infection. But most of them returned to normal within a week. Disturbances of ventilation analysed with Xe radio spirometry remained abnormal several months after the attack. It is not possible to say whether the impairment cause a transient derangement or can cause permanent residual damage with risk of chronic obstructive lung disease in later life. Sabeto et al[11] also showed that after *Mycoplasma* infection, lung function could be deranged for a variable period of time, when they followed up children with *Mycoplasma* infection. Our study failed to show significant abnormality between the groups, may be due to lack of follow up of the patients, intense treatment age characteristics of the group and since most of the previous studies for lung function were done in children. Studies by Kraft et al,[6] Nicholson et al[19] and Kuppeveld[22] have shown that acute exacerbations of asthma and chronic nature of asthma may be due to the colonisation of the organism in the lower respiratory tract and may cause culture and serology to be negative and PCR to be positive.

In our study we also found that 22% of *Mycoplasma* isolated sputum, also contained secondary pathogens like streptococcus pneumonia and H Influenza by Gram stain. But pyogenic cultures were negative for all patients suggesting that other sensitive tests for pyogenic organisms, viral cultures, serology for suspected pathogens and even PCR is needed for measuring exact role of *Mycoplasma* in the pathogenesis of asthma exacerbation and chronicity.

On viewing X ray PNS for sinusitis, there was no association between presence of sinusitis and *Mycoplasma* pneumonia incidence suggesting that sinusitis may be a nonspecific finding. Asthma itself is associated with higher incidence of sinusitis as suggested by Kraft et al.[23] Studies by Kraft et al[23] had shown that upper respiratory tract infections could be caused by *Mycoplasma* infections but ours was a lower respiratory tract specimen, simultaneous examination with nasal swab culture may give conclusive evidence for these findings.

In the *Mycoplasma* positive group, 16% of them had exacerbation of asthma. Since the pyogenic culture were negative for all these patients, we presume that the exacerbations were caused by *Mycoplasma* aitself and our finding of increased incidence of *Mycoplasma* and acute exacerbation of asthma is comparable with other studies done by Seggev et al[12] and Lieberman et al[16] even though viral cultures were not done on any of our patients. Most of the earlier studies by Huhti et al,[24] Berkowich wet al,[25] Tipirineni et al[18] for isolation of *Mycoplasma* were done using serologic methods, since *Mycoplasma* infection in the acute phase can raise the immunoglobulin levels (total Ig E and *Mycoplasma* specific Ig E). The incidence from these previous studies were in the range of 21-32%. Recent studies by Buck et al.[26] Kuppeveld[22] and Kraft et al[6] by polymerized chain reaction for *Mycoplasma* isolation indicates that PCR can be positive for longer periods than culture or serology. So, they proposed that asthmatics appear to be colonised by or chronically infected with *Mycoplasma* in the lower airways without the activation of the immune system and or the *Mycoplasma* can cause immune paresis making serology negative and causing inflammation to continue without check.

One study by Kraft et al[6] which compared PCR, culture and serology has shown that by PCR *Mycoplasma* was isolated in 555 whereas all of them were negative for culture and serology. (study done on chronic stable adult asthmatics). Other studies by Kuppeveld[22] and Kok T[9] showed an increased isolation of *Mycoplasma* from sputum by culture by about 14%. In our study by isolating *Mycoplasma* by sputum culture, most patients were having chronic asthma (89%) without exacerbation, showed an isolation rate of 16% which is higher than expected. The disparity may be accounted by geographical distribution and survival characteristics, use of transport media, age characteristics of the patients. We were not able to find similar study in our part of the country. So further studies are to be carried out especially in the same geographical area to prove or disprove your findings.

CONCLUSIONS

Mycoplasma was found in 18% of asthmatic patients. Parameters like daytime symptom score and eosinophil count showed statistically significant difference between the *Mycoplasma* positive and negative group, whereas age, sex, family history of asthma, X Ray PNS, Gram stain and IgE levels did not show any statistically significant difference between the two groups.

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