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COMPARISON OF IgM ELISA AND CULTURE IN THE DIAGNOSIS OF COMMUNITY ACQUIRED PENUMONIA DUE TO *MYCOPLASMA PNEUMONIAE* IN A TERTIARY CARE HOSPITAL

Medical Science

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ABSTRACT

Community Acquired Pneumonia (CAP) is a lower respiratory tract parenchymal lung infection which continues to be a major health problem. Identifying the cause remains a challenge. Although aetiology varies per geographic region, of all the etiological agents of CAP, atypical respiratory bacteria are increasingly being recognised as emerging pathogens. Atypical bacteria rank as third or fourth leading organisms causing CAP, being present in about 20-40% of all CAP patients, often as co-pathogens. The most common organisms are *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila.* Among them, *M. pneumoniae* is one of the leading causes of CAP. This study is an attempt to compare results of IgM ELISA and culture in the diagnosis of CAP due to *M. pneumoniae.* A detailed clinical history was taken from 100 patients above 18 yrs of age with symptoms of LRTI. Throat swab was collected in PPLO broth. 3-5 ml of blood was collected in a plain bulb for detection of IgM antibodies against *M. pneumoniae*. *Mycoplasma pneumoniae* could not be isolated on culture from any of the throat swab samples. Serum samples of all the patients were tested for the presence of *Mycoplasma pneumoniae* IgM antibodies by ELISA and were found positive in 10 samples (10%).

KEYWORDS

Community Acquired Pneumonia, Mycoplasma pneumoniae, Serology, Culture

INTRODUCTION

Community Acquired Pneumonia is one of the most important causes of morbidity and mortality worldwide.[1] Mortality data specific to the community acquired pneumonia is not available for the Indian population, however, lower respiratory tract infections are responsible for around one-fifth of the deaths caused due to infectious diseases in India and pneumonia is a major culprit.[2] Although aetiology varies per geographic region,[3] of all the etiological agents of CAP, atypical respiratory bacteria are increasingly being recognised as emerging pathogens.[4] Atypical bacterial pneumonia is caused by atypical organisms which are not detected on Gram stain and cannot be cultured using standard methods, and characterized by a symptom which includes headache, low-grade fever, cough, and malaise. Atypical bacteria rank as third or fourth leading organisms causing CAP, being present in about 20-40% of all CAP patients, often as co-pathogens.[5] The most common organisms are *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila.* Among them, *M. pneumoniae* is one of the leading causes of CAP.[6] Although treatment is straight forward, proving *Mycoplasma pneumoniae* infection in specific patients is a complex task as it is difficult to culture and is not normally detected in bronchial secretions, thus serology is the principal method for laboratory diagnosis.[7]

MATERIAL AND METHODS

Study Design:

The study was an observational study carried out in the Department of Microbiology in collaboration with the Medicine department at a tertiary care hospital in Western Maharashtra. Ethical clearance was taken, and the duration of the study was from January 2018 to December 2018.

Study Population: 100 clinically diagnosed patients of CAP

Inclusion Criteria: All adults (>18 yrs) with

- Signs and symptoms with LRTI (cough with or without expectoration, dyspnea, pleuritic chest pain, crackles on auscultation)
- New focal chest signs on physical examination
- At least one systemic feature (sweating, fever, shivering, aches or pains)
- No other explanation of the illness

Exclusion Criteria:

- Patients with immunosuppression
- An emerging alternative diagnosis during follow-up
- Pneumonia secondary to tuberculosis
- History of hospitalization within the previous 10 days
- Ventilator-associated pneumonia

Study Procedure:

- Detailed history along with general physical examination findings, clinical findings and radiological findings of all patients were collected.
- Specimen was collected and swab was transported to laboratory in 2 ml of PPLO broth.
- The broth was incubated at 37^{0}C and was observed for colour change after a week.
- After one week, in presence of colour change to yellow, broth was subcultured on PPLO agar.
- 100 l of the broth using a micropipette were dispersed on to the agar medium.
- PPLO agar was incubated at 37^{0}C and observed for presence of colonies every week for four weeks in an inverted position under microscope.
- In presence of the colonies, the block of agar is cut and stained with Diene's stain to confirm the presence of *Mycoplasma pneumoniae*.
- In absence of obvious colour change, blind subculture to agar was performed after 1st and 3rd week of incubation.

Serum IgM ELISA test :

3-5 ml of blood was collected in a plain bulb for detection of IgM antibodies against *Mycoplasma pneumoniae* by NOVATEC Mycoplasma pneumoniae IgM ELISA. Serum was obtained after centrifugation of blood sample. The test was conducted as instructed in the kit manual.

Interpretation :

- Samples were considered **POSITIVE** if the absorbance value was higher than 10% over the cut-off.
- Samples were considered **NEGATIVE** if the absorbance value was lower than 10% below the cut-off.
- Samples with an absorbance value of 10% above or below the cut-off were not considered as clearly positive or negative rather be considered to fall under **GREY ZONE**.
- It was recommended to repeat the test again 2-4 weeks later with a fresh sample. If the results in the second test were again in the grey zone, then sample had to be considered negative.

Statistical Analysis :

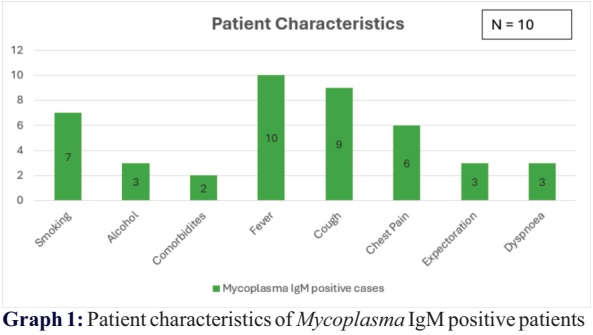
All the data was entered using the SPSS Statistical software package, Version 23.0.0.0 (IBM Corporation). Continuous variables were expressed as mean ± standard deviation. Pearson chi-square test was done for categorical variables. Pearson correlation test was done to find a correlation. A 2-sided P value ≤ 0.05 was considered statistically significant.

RESULTS

- *Mycoplasma pneumoniae* could not be isolated on culture from any of the throat swab samples.
- Serum samples of all the patients were tested for the presence of *Mycoplasma pneumoniae* IgM antibodies by ELISA and were

found positive in 10 samples (10%).

- Of these 10 patients, 6 were male and 4 were female. No significant association of *Mycoplasma pneumoniae* infection with the sex of the individual could be found (p value is 0.375, i.e. >0.05).
- The mean age of the patients was 48.9 years with a standard deviation of 15.19 years. There was no statistical significant association seen with *Mycoplasma pneumoniae* infection and age (p value is 0.546, i.e. >0.05).
- A total of 7 out of 10 gave history of smoking and 3 admitted to alcohol use. Two patients had associated co-morbidities, one had diabetes and one had COPD. None of these findings were statistically significant.
- All the patients presented with fever, 9/10 had cough and 6/10 complained of chest pain. Statistically significant differences were obtained for fever (p value is 0.04) and cough (p value – 0.01) but not for chest pain (p value – 0.113).
- Dyspnoea and expectoration were seen in 3/10 cases. They were not statistically significant.
- Predominant radiological finding was interstitial pneumonia seen in 50% of cases, 40% had lobar pneumonia and there was one case of bronchopneumonia.
- **BACTERIAL CO-INFECTION IN IgM + PATIENTS :** Co-infection with other bacteria was seen in 60% of the patients who came positive for *Mycoplasma* IgM, 3 (30%) had *Klebsiella pneumoniae* isolated from their sputum, *Streptococcus pneumoniae* was isolated in 2 (20%) cases and *Enterobacter aerogenes* in one patient (10%).



Graph 1: Patient characteristics of *Mycoplasma* IgM positive patients

DISCUSSION

In our study, 10 patients tested positive for *Mycoplasma pneumoniae* IgM antibody by ELISA. Of these 6/10 (60%) were male and the rest (40%) were female. No significant association of *M. pneumoniae* infection with the sex of the individual could be found (p value is 0.375, i.e. >0.05). The mean age of the patients was 48.9 years with a standard deviation of 15.19 years. There was no statistical significant association seen with *M. pneumoniae* infection and age (p value is 0.546, i.e. >0.05). Elkolaly et al.[8] in a study conducted in Egypt in 2019 had similar findings.In the present study, 70% (7/10) gave history of smoking and 30% admitted to alcohol use. Only 20% had associated co-morbidities. None of these were statistically significant. Comparison of presenting symptoms between *M. pneumoniae* infected and non-infected patients revealed that fever (100%), cough (90%), chest pain (60%) were predominant findings. Statistically significant differences were obtained for fever (p value 0.04) and cough (p value 0.01) but not for chest pain (p value 0.113). These findings are similar to Dash S et al. [New Delhi, 2018] [9].

Radiological features predominant in this study was interstitial pneumonia, seen in 50% of the cases. 40% had lobar pneumonia and there was one case with bronchopneumonia. Dash S et al. [New Delhi, 2018] [9] reported similar findings.In none of the serologically positive samples, *M. pneumoniae* could be isolated on culture. This is in accordance with the findings of Basil MV et al.[10] conducted in 2009 in Delhi where they studied 100 patients and the seroprevalence of *M. pneumoniae* was 16% by ELISA and in none of the cases the organism could be isolated on culture.

Our study was limited by the fact that being a tertiary care hospital, the patients arriving here had already been administered two-to-three doses of antimicrobials before collection of samples due to the presence of clinical manifestations which may explain culture negative results in serology-positive patients.

Seroprevalence of *M. pneumoniae* in present study was 10%. In another study conducted in Mumbai in 2015, Bhattacharjee M et al.[11] reported seroprevalence of *Mycoplasma pneumoniae* to be 12.66%.

CONCLUSION

Serological tests are more sensitive for the detection of acute *M. pneumoniae* infection compared to culture. Culture being expensive, laborious and time consuming, is rarely performed and not recommended for routine diagnosis due to the prolonged turnaround time, requirement of specialized expertise and low sensitivity. Enzyme immunoassays are the most widely used serological tests and they comparable in sensitivity to PCR, provided that sufficient time has elapsed since infection for antibody to develop and patient has a functional immune system.

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Conflict Of Interest: None

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