Colonization of mycoplasma in the upper respiratory tract of AIDS patients with pulmonary symptoms in Chennai, India


Department of Microbiology, Dr ALM Postgraduate Institute of Basic Medical Sciences, University of Madras & *Government Hospital of Thoracic Medicine, Chennai, India

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**Background & objectives**: Mycoplasmas have been implicated in causing minor to severe respiratory infections in man. Mycoplasmas are considered to act as cofactors in patients with AIDS. A preliminary study was conducted to isolate mycoplasmas from sputum specimens of AIDS patients and non-HIV patients with underlying pulmonary symptoms and signs.

**Methods**: A total of 130 sputum samples (100 from AIDS patients and 30 from non-HIV) were cultured on standard pleuropneumonia-like organisms (PPLO) glucose agar up to 3 wk. The plates were examined for the presence of fried-egg colonies characteristic of *Mycoplasma*. Subsequently the plates were stained using Diene’s stain. Sputum specimens from the AIDS patients were also screened for other bacterial pathogens.

**Results**: Mycoplasmas were detected from 36 (36%) of the AIDS patients and only 5 (16.6%) of the non HIV control individuals with underlying pulmonary symptoms. Data on the detection rates of other microorganisms from the AIDS cases were also analysed.

**Interpretation & conclusion**: This preliminary study provided supportive evidence that mycoplasma colonized in upper respiratory tract of individuals with AIDS to a larger extent than that of the non HIV subjects with pulmonary symptoms. Further studies need to be done to characterize mycoplasma isolates to species level.

**Key words** Diene’s stain - fried-egg morphology - mycoplasma

Mycoplasmas are commensals causing self-limiting and clinically unimportant infections in human beings\(^1\). Recent isolation of these organisms from adults with AIDS suggests that mycoplasmas might act as cofactors in patients infected with human immunodeficiency virus (HIV)\(^2,4\). The four species of mycoplasmas identified as being associated with AIDS include *Mycoplasma fermentans*, *M. pirum*, *M. penetrans* and *M. genitalium*. But it is worthwhile to note that mycoplasmal infection is highly significant clinically in AIDS, even if it is merely one more example of an opportunistic infection\(^5,7\).
Mycoplasmas cause a variety of clinical conditions in the immunocompromised individuals. The gold standard laboratory method for diagnosing mycoplasma infection has been culture. Hjordis isolated *M. pneumoniae* from respiratory specimens from AIDS patients by culture. Ainsworth et al have detected the presence of *M. fermentans* in the respiratory tract of 27 per cent of the HIV population. A detection rate of 12.5 per cent of mycoplasmas has been documented from bronchoalveolar lavage specimens of HIV infected patients which indicated that AIDS patients might be more often colonized or infected by mycoplasmas than HIV-negative patients or other immunocompromised persons. In another study mycoplasmas have been shown to colonize HIV-positive patients to a larger extent than HIV-negative individuals. Teel et al have reported that mycoplasmas colonized the respiratory tracts of 28 per cent of HIV-positive and 10.5 per cent of HIV-negative patients. Mycoplasmas are known to cause damage to the respiratory epithelium and cause respiratory infections in man. We undertook this study to isolate mycoplasmas from sputum specimens of AIDS patients and HIV-negative patients with pulmonary signs and symptoms in Chennai, Tamil Nadu, India.

**Material & Methods**

Sputum specimens collected from 130 patients (100 AIDS and 30 non-HIV) admitted to Government Hospital of Thoracic Medicine, Chennai during May 2001 to January 2003 were cultured for mycoplasmas. The point system using the PAHO/CARACAS, 1989 regulations was used to assess the AIDS-status in the selected patients. The patients in non-HIV group were selected only after they tested negative for HIV-1 antibodies using HIV-1 Western blotting (Genetic Systems™ HIV-1 Western blot, USA). Patients complaining cough, malaise, headache, chilliness, sore throat, chest discomfort, nasal symptoms, myalgias, fever, rales, wheezes, and pharyngeal erythema were considered for the study after obtaining informed consent and prior approval by the ethics committee of the institute. The patients were not included consecutively due to difficulties in follow up. AIDS patients receiving highly active anti-retroviral treatment (HAART) were excluded from the study.

Collection of sputum specimens was carried out as suggested by Brahmadathan et al and mycoplasma culture as described by Velleca et al. Briefly, after transportation to the laboratory, sputum specimens were inoculated on pleuropneumonia-like organisms (PPLO) glucose agar (HiMedia, Mumbai, India) using sterile alginate swabs. The plates were incubated aerobically at 37°C with 5 per cent CO₂ (Gas-pak jars, BBL Microbiology, Cockeysville, USA) for up to 3 wk and their surfaces were periodically observed using an inverted microscope (Nikon, Japan) for the presence of colonies with a fried-egg appearance. The positive plates were located for colonies and about 1 cm² of the agar lodging the colony was cut with a sterile scalpel and transferred to broth for subculturing and incubated. Broth cultures were frequently examined for thread-like sediments and pH change. One ml of culture positive broth was passed through a 0.2 µm pore size acrodisc filter (HiMedia, Mumbai, India). The filtrate was inoculated into 5 ml of broth and agar. Plates that showed fried-egg colonies were stained using Diene’s stain prepared manually. Identification of Mycoplasma colonies was done based on biochemical tests to identify mycoplasmas up to the preliminary genus. Identification procedures recommended (i) colony appearance (fried-egg appearance), (ii) filterability (isolates can be passed through a 0.2 µm pore size filter), (iii) absence of reversion in antibiotic-free growth medium, and (iv) biochemical characteristics (glucose utilizing). Mycoplasma cultures in fluid medium were aliquoted into samples of 1 ml in sterile vials and stored at -70°C.

Sputum specimens from AIDS cases were simultaneously screened for other organisms by culture on blood agar MacConkey agar and Sabouraud’s dextrose agar (HiMedia, Mumbai, India). Ziehl-Neelson’s acid-fast staining (HiMedia,
Mumbai, India) was performed to detect acid fast bacilli (AFB). The non-HIV specimens were screened only for mycoplasmas due to certain technical inabilities.

Statistical analysis: Data were analysed using Pearson’s Chi square test.

Results & Discussion

Patients with AIDS are at risk of respiratory tract infections. Of the 130 patients who underwent sputum culture, 100 were confirmed as AIDS patients, and 30 were seronegative for HIV-1 antibodies and hence considered as non-HIV controls. The mean age of patients in the AIDS group was 40.4 yr (40 ± 2 yr), with a range of 21 to 60 yr, and ratio of male to female was 2.1:1. The HIV-negative group had a mean age ± yr (22 to 82 yr) and men outnumbered women 1.5:1.

Of the 130 sputum specimens cultured, 41 (36 from AIDS group, 5 from HIV-negative group) grew mycoplasmas (31.5%). Mycoplasma positivity was 36 per cent in AIDS group and 16.6 per cent in HIV-negative group, the difference was statistically significant (P<0.05).

Sloot et al\textsuperscript{11} have reported that mycoplasmas were found to colonize 12.5 per cent of the HIV infected cases compared to <0.9 per cent HIV-negative patients. In another study, 87 per cent HIV-positive patients tested positive for mycoplasmas compared to only 20 per cent from the HIV-negative controls\textsuperscript{8}. We have identified mycoplasmas more frequently from the sputum of AIDS patients (36%) than from the non-HIV controls (16.6%) (OR=2.81 and 95% CI = 0.91 to 10.16). However, our study failed to correlate mycoplasma positivity with respect to age (Table I).

Culture for other microbiological agents in AIDS patients revealed \textit{Candida} spp., \textit{Staphylococcus} spp., \textit{Streptococcus} spp., \textit{Klebsiella pneumoniae}, \textit{Pseudomonas aeruginosa} and \textit{S. pneumoniae} (Table II). \textit{P. aeruginosa} (10.7%), \textit{S. pneumoniae} (12.5%) and \textit{K. pneumoniae} (32.8%) were found to be more in mycoplasma negative cases than the mycoplasma positive ones. Further, 2 (3.1%) isolates of diphtheroids were detected from the mycoplasma negative AIDS cases. Ten patients each in mycoplasma positive and the negative groups were positive for AFB which corroborated with the detection of \textit{Mycobacterium} spp. in respiratory specimens of HIV infected patients in earlier studies\textsuperscript{12}. In addition to mycoplasmas, a possible pulmonary pathogen was isolated from all the AIDS patients.

Mycoplasma culture is notoriously difficult and laborious. But direct isolation is considered the gold standard. So in this study we attempted to isolate mycoplasmas from sputum specimens of AIDS patients characteristic fried-egg colonies of mycoplasmas (Fig. 1) on culture and after staining

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>No. of cases</th>
<th>No. of culture positives for mycoplasma</th>
</tr>
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<tbody>
<tr>
<td>AIDS (n=100)</td>
<td>Non-HIV (n=30)</td>
<td>AIDS (n=36)</td>
</tr>
<tr>
<td>21-30</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>31-40</td>
<td>42</td>
<td>6</td>
</tr>
<tr>
<td>&gt;40</td>
<td>24</td>
<td>12</td>
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<table>
<thead>
<tr>
<th>Organism detected</th>
<th>Mycoplasma positive cases (n = 36)</th>
<th>Mycoplasma negative cases (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Candida} spp.</td>
<td>23 (63.8)</td>
<td>48 (75)</td>
</tr>
<tr>
<td>\textit{Staphylococcus} spp.</td>
<td>18 (50)</td>
<td>16 (25)</td>
</tr>
<tr>
<td>\textit{Streptococcus} spp.</td>
<td>13 (36)</td>
<td>18 (28)</td>
</tr>
<tr>
<td>\textit{Klebsiella pneumoniae}</td>
<td>7 (19.4)</td>
<td>21 (32.8)</td>
</tr>
<tr>
<td>\textit{Streptococcus pneumoniae}</td>
<td>1 (2.7)</td>
<td>8 (12.5)</td>
</tr>
<tr>
<td>\textit{Pseudomonas aeruginosa}</td>
<td>1 (2.7)</td>
<td>7 (10.9)</td>
</tr>
<tr>
<td>Acid-fast bacilli</td>
<td>10 (27.7)</td>
<td>10 (15.6)</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>0 (0)</td>
<td>2 (3.1)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the percentage
associated mycoplasmas) from respiratory specimens of AIDS patients assumes significance as they are considered as definitive proof of infection. We could not characterize mycoplasma isolates to species level in this study. There are reports on the isolation of AIDS-associated mycoplasmas from HIV-positive adults. These observations plus our present observations suggested that mycoplasmas might be a co-pathogen in the pathogenesis of AIDS, which implies that infections with mycoplasma requires both HIV infection and associated immunodeficiency.

In conclusion, our study showed that mycoplasmas were more commonly isolated from AIDS patients than HIV-negative controls with pulmonary symptoms. Future attempts need to be made to study the species of mycoplasma isolates and their role in the progression of HIV-disease.

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References


Reprint requests: Dr Usha Anand Rao, Department of Microbiology, Dr ALM Postgraduate Institute of Basic Medical Sciences University of Madras, Taramani Campus, Taramani, Chennai 600113, India e-mail: drushaanand@yahoo.com