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Original Article

Microbiology Section

Detection of *Cytomegalovirus* in Bronchoalveolar Lavage Fluid from HIV-Positive Individuals with Community Acquired Pneumonia

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ABSTRACT

Introduction: *Cytomegalovirus* (CMV) pneumonia is one of the frequent viral pneumonia reported in persons with HIV infection. Knowledge of pulmonary CMV infection is important for deciding appropriate diagnostic strategies. However, there is scanty literature addressing the role of CMV aetiology among HIV positive individuals presenting with Community Acquired Pneumonia (CAP) using Bronchoalveolar Lavage (BAL) samples from India.

Aim: To detect CMV in BAL fluid from HIV-positive individuals presenting with CAP.

Materials and Methods: This cross-sectional study was conducted using 107 archival BAL samples collected from consecutive HIV-positive patients presenting with CAP as per the Indian Chest Society and National College of Chest Physicians guidelines at the Department of Chest and Tuberculosis, Sassoon General Hospitals, Pune, India. The samples were tested for CMV by Polymerase Chain Reaction (PCR) targeting the IRL11 region at the National AIDS Research Institute, Pune.

Results: Of the 107 BAL samples tested, 8 (7.4 %) were positive for CMV, while CMV was the sole pathogen in 5 (4.7%) cases. Co-infection with other pathogens was seen in 3 patients and *Mycobacterium tuberculosis*, *Pneumocystis jiroveci* and *Streptococcus pneumoniae* were the co-pathogens. Five patients had fatal clinical outcome of which three had CMV as the sole pathogen.

Conclusion: Ours is the first study to detect *Cytomegalovirus* (CMV) in bronchoalveolar lavage samples from HIV-positive individuals presenting with community acquired pneumonia from India and indicates the need for further multicentre studies to understand pulmonary CMV infection, which will eventually help in designing appropriate diagnostic strategies and therapeutic interventions.

Keywords: Antiretroviral treatment, *Pneumocystis jirovecii,* Polymerase chain reaction

INTRODUCTION

The lungs are a principal target of HIV-associated complications and opportunistic pneumonias are major causes of morbidity and mortality among these individuals [1]. *Cytomegalovirus* (CMV) pneumonia is one of the frequent viral pneumonia reported in people living with HIV infection (PLHIV), though retinitis and gastrointestinal disease dominate the clinical manifestations [1,2]. The role of CMV as a primary pulmonary pathogen has been questioned [3]. Establishing the diagnosis of CMV pneumonia in PLHIV is difficult because; the clinical abnormalities are not distinctive, CMV is often recovered from pulmonary secretions in the absence of histologic evidence of disease and CMV is likely to coexist with other pulmonary pathogens [4,5]. Knowledge of pulmonary CMV infection is important for designing diagnostic strategies and planning subsequent therapeutic interventions. There is sparse data on pulmonary CMV infection among HIV-positive individuals from India as testing for CMV is rarely done. The only literature on pulmonary CMV infection among PLHIV from India is the autopsy report by Lanjewar DN et al., where the prevalence of pulmonary CMV infection of 7% was reported [6].

Thus, we proposed the present study to detect CMV in Bronchoalveolar Lavage (BAL) fluid samples from HIV-positive individuals presenting with Community Acquired Pneumonia (CAP) from Pune, India.

MATERIALS AND METHODS

A total of 107 archival BAL samples collected as part of a previous study [7] to detect *Pneumocystis jirovecii* infection among HIV-positive patients were used in the present study. The study was approved by Institutional Ethical Committees of the BJ Government College and Sassoon General Hospitals, Pune and the National AIDS Research Institute (NARI), Pune, India.

Of these 107 patients, 67 (62.6%) were males and 40 (37.4%) were females, with median age of 39 years (range 18-70), median CD4+ count of 257 cells/mm^3 (range, 17–1661), while 56 (52.3%) patients were receiving Antiretroviral Treatment (ART).

The samples were collected at the Department of Chest and Tuberculosis, Sassoon General Hospitals, Pune, India. Inclusion criteria for the patients were presence of at least one major clinical criteria (cough, sputum production and fever >37.8°C) or two minor criteria (pleuritic chest pain, dyspnoea, altered mental state, total leucocyte count of ≥12,000/µl or sign of pulmonary consolidation on examination) with a new pulmonary infiltrate/shadow on chest X-ray suggestive of pneumonia [8]. Patients were non-responsive to initial empirical antibiotic therapy.

The exclusion criteria were patients who were less than 18 years of age, reporting hospitalization within seven days, critically ill and those refusing to consent.

The laboratory processing of samples for detection of CAP aetiologies was done at the Department of Microbiology, NARI, Pune. Bacterial and mycobacterial identification was performed using standard microbiological techniques [9], while atypical bacteria and *Pneumocystis jirovecii* were detected as described earlier [7,10].

The storage of residual BAL samples was done at the Department of Microbiology, National AIDS Research Institute, Pune, India.

These samples were used for detection of CMV DNA by PCR. CMV infection was defined as patients suspected of having pneumonia with positive CMV DNA detection in BAL [11].

Sample Preparation and PCR Amplification of CMV

DNA was extracted from 300 µl of BAL fluid according to the manufacturer's instructions using the QIAamp DNA mini kit (Qiagen). Water was extracted following every fifth sample to rule out carry over contamination. CMV PCR was performed with primers CP15 F- 5' GTACACGCACGCTGGTTA CC 3' and CM3 R-5' GTAGAAAGCCTCGACATCGC 3' targeting the IRL11 region [12]. PCR was performed in 50 µl containing 5 µl 10X buffer, 2.5 mM $MgCl_2$, 200 mM each dNTP, 10 pmol each primer, 0.5 U Taq polymerase and 5 µl of DNA, with the remaining volume made up with sterile distilled water. Amplification was performed by initial denaturation at 94°C for one minute, followed by 30 cycles of 15 seconds at 94 °C, 20 seconds at 65 °C and 30 seconds at 72°C, with a final extension at 72°C for 10 min in a thermal cycler (GeneAmp PCR System 9700, AB Biosystems).

All reaction products (256 bp) were separated by electrophoresis on a 2% agarose gel for one hour at 100 V at room temperature in Tris base, acetic acid and EDTA buffer stained with ethidium bromide and visualized using a gel documentation system (Bio-Rad) as shown in [Table/Fig-1]. Known positive (obtained from the National Institute of Virology (NIV), Pune) and negative controls (sterile distilled water) were included in each run.

STATISTICAL ANALYSIS

Statistical analysis was done by using the SPSS statistical package version 15.0. Fisher's-exact test and Mann-Whitney U test were used to determine the association of CMV status with the different characteristics. Results with p-value <0·05 were considered as statistically significant.

RESULTS

Of the 107 BAL samples, 8 (7.4 %) samples were positive for CMV DNA PCR, while CMV was the sole pathogen in 5 (4.7%) cases. The characteristics of CMV positive patients are presented in [Table/Fig-2]. Of the eight patients, five were males and three were females, with median age of 37.5 years (range 23–46) and median CD4 count of 75 cells/mm^3 (range, 63–175). Three patients were on antiretroviral treatment, while two had previous history of prophylaxis with cotrimoxazole.

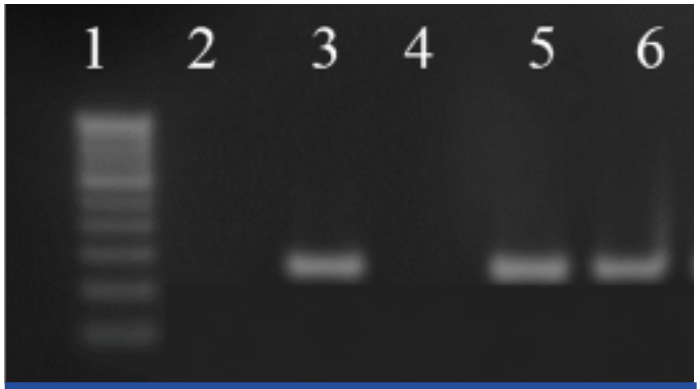
The symptoms of cough, fever and dyspnea were present in all individuals, while radiological findings of bilateral interstitial shadows and consolidation were primarily observed. The patients received antibiotics and/or antitubercular drugs depending on the laboratory diagnosis. Co-infection with other pathogens was seen in 3 (37.5%) patients and *Mycobacterium tuberculosis*, *Pneumocystis jiroveci* and *Streptococcus pneumoniae* were the co-pathogens. Five of the eight (62.5%) patients had fatal clinical outcome, of which three had CMV as the sole pathogen.

The characteristics of CMV-positive patients (n=8) were compared with patients having other microbial aetiologies (n=82) [Table/Fig-3]. The patients with unidentified aetiologies (n=17) were not included in the analyses. CMV-positive has significantly greater multilobar involvement as compared to patients having other aetiologies (p=0.042). There were no statistically significant differences between the groups in other characteristics like age (p=0.406), gender (p>0.999), ART status (p=0.480), co-morbidities (p>0.999), presence of mono/poymicrobial aetiologies (p=0.195), and CD4 count (p=0.278) and mortality (p=0.111).

DISCUSSION

CMV has long been recognized as a cause of pneumonia in the immunocompromised host [1]. Detection of pulmonary CMV infection in HIV-positive individuals is important because CMV replication is associated with accelerated HIV disease progression and as well as with increased risk of CMV end-organ disease. Likewise there are specific therapy recommendations for the prevention and treatment of CMV disease in immunocompromised hosts [13]. Treatment with intravenous ganciclovir, foscarnet and more recently with valganciclovir is usually instituted. Severe CMV disease or CMV end-organ disease can be prevented by timely detection of CMV infection and instituting ART and appropriate therapy [2]. The definitive diagnosis of CMV pneumonia depends on documentation of CMV infection in lung tissue; however, performing lung biopsy in PLHIV is highly risky.

Recent literature suggests the utility of BAL as a less invasive option to access lung pathology and to aid in the diagnosis of CMV pneumonitis using molecular methods [14]. Among bone-marrow and organ transplant recipients, the detection of CMV in BAL is reported to be highly predictive of the development of CMV pneumonia [15-17]. Recently, Kaur A et al., has reported a higher prevalence of CMV (21%) among immunocompromised patients other HIV infection has suggested that CMV DNA detection in BAL can give useful information if done in clinically suspected immunocompromised patients [18].



[Table/Fig-1]: Detection of *Cytomegalovirus* DNA by polymerase chain reaction. Lane 1: molecular ladder (100bp); Lane 2: negative control; Lane 3: positive control, Lane 4: sample negative for CMV; Lanes 5, 6: samples positive for CMV (256bp).

Patient number	Age (years)	Gender	CD4 count	ART status	Co-pathogen	Cotrimoxazole prophylaxis	X-Ray findings	Clinical outcome
1	42	Male	83	No	*Mycobacterium tuberculosis*	No	lt l/l consolidation	Died
2	30	Female	65	No	No	No	b/l infiltrates	Died
3	40	Female	67	No	No	No	b/l shadows	Cured
4	27	Male	73	Yes	*Pneumocystis jiroveci*	Yes	rt l/l consolidation	Cured
5	46	Male	63	No	No	No	b/l infiltrates	Died
6	36	Male	77	N0	*Streptococcus pneumoniae*	No	b/l infiltrates	Died
7	39	Female	175	Yes	No	Yes	lt l/l consolidation	Cured
8	23	Male	103	Yes	No	No	b/l infiltrates	Died

[Table/Fig-2]: Characteristics of patients with *Cytomegalovirus* in bronchoalveolar lavage fluid.
lt-left, rt-right, l/l-lower lobe, b/l-bilateral, ART-antiretroviral treatment

Variable		CMV present (n=8)	Other aetiology (n=82)	p-value
Age (years) (Median with range)		37.5 (23-46)	39 (18-62)	0.406
Gender	Male	5 (62.5%)	52 (63.4%)	>0.99
	Female	3 (37.5%)	30 (36.6%)	
CD4 count (cells/mm^3) (Median with range)		75 (63-175)	100 (74-661)	0.278
Antiretroviral treatment	Yes	3 (37.5%)	43 (52.4%)	0.480
	No	5 (62.5%)	39 (47.6%)	
Aetiology	Monomicrobial	3 (9.1%)	8 (57.1%)	0.195
	Polymicrobial	30 (90.9%)	6 (42.9%)	
Co-morbidities	Present	1 (12.5%)	16 (19.5%)	>0.99
	Absent	7 (87.5%)	66 (80.5%)	
Lung involvement	Monolobar	3(37.5%)	61 (74.4%)	0.042
	Multilobar	5 (62.5 %)	21 (25.6%)	
Clinical outcome	Cured	3 (37.5%)	57 (69.5%)	0.111
	Died	5 (62.5%)	25 (30.5%)	

[Table/Fig-3]: Comparison of characteristics of patients with CMV aetiology verses other microbial aetiologies.

In the present study, CMV infection was detected in 7.4% BAL samples from HIV-infected patients with pulmonary symptoms. The CMV prevalence in this study concords with the prevalence reported in the autopsy report from India [6]. Variable prevalence rates of pulmonary CMV infection have been reported globally. Autopsy studies conducted in HIV/AIDS patients have reported the presence of CMV infection in 7%-81% cases [19], while studies using BAL have reported CMV prevalence up to 72% [20]. The differences in CMV prevalence observed in various studies can be attributed to the different geographical location and the diagnostic methods used, including histopathology, culture, antigenemia and PCR assays [14]. In accordance with previous studies co-infection with other pathogens was observed [18,21,22].

CMV-positive patients had significantly greater multilobar involvement as compared to patients with other aetiologies. This can be attributed to the cytopathogenic effects of CMV causing diffuse alveolar damage [23]. Pulmonary CMV involvement is a sign of wide viral dissemination and is reported to be associated with an elevated mortality rate [1,2]. This explains the relatively high mortality (62.5%) observed in patients with CMV, further endorsing the need for timely detection of CMV infection.

LIMITATION

Ours was an exploratory study to detect CMV infection in HIV-positive individuals with pneumonia conducted in a single centre and hence the results may not be easily generalizable to the entire country. No differentiation between endogenous reactivation and exogenous infection as the cause of the active infection could be made.

CONCLUSION

Ours is the first study to detect CMV in bronchoalveolar lavage samples from HIV-positive individuals presenting with community acquired pneumonia from India. The results indicate that CMV should be suspected in pneumonia patients non-responsive to initial empirical treatment and with multi-lobar radiological involvement to avert further complications. The need for conducting larger prospective multicentre studies to confirm our findings and to understand pulmonary CMV infection among HIV-infected individuals is warranted, which may eventually help in designing appropriate diagnostic strategies and therapeutic interventions.

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