Original Article

Profile of pneumocystis infection in a tertiary care institute in North India

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Abstract

Context: *Pneumocystis jirovecii* pneumonia (PcP) is still remains a common opportunistic disease in human immunodeficiency virus (HIV) infected individuals. Study on PcP in developing countries are scarce. **Aims:** To study the occurrence of *P. jirovecii* infection in clinically suspected individuals in a tertiary care institute. **Settings and Design:** Retrospective study conducted in a tertiary care hospital. **Materials and Methods:** Two years data regarding respiratory sample analysis, HIV status, and cluster of differentiation 4 (CD4) cell count of clinically suspected pneumocystis infection patients with known/unknown HIV status were analyzed. **Results:** Data of 45 eligible patients were analyzed. The majority of the patients were male (between 21 and 50 years of age). Total 26 (57.7%) patients were HIV reactive, of which 14 had CD4 count of <200 cells/mm^3. 20 patients (9 HIV reactive and 11 unknown HIV status) were confirmed with pneumocystosis by direct fluorescent antibody (DFA) staining. Four of 14 HIV reactive individuals who had CD4 count of <200 cells/mm^3 and 5 of 12 HIV reactive individuals who had CD4 count of >200 cells/mm^3 were positive for pneumocystosis. **Conclusions:** Pneumocystis pneumonia is still prevalent in North India and is mainly affecting patients in economically productive and sexually active age group. To diagnose pneumocystosis, DFA is an easily available method in resource-limited settings. Appreciating the actual HIV or immunodeficiency status and the CD4 profile of an individual with symptoms of pneumocystis infection will help the clinicians in early diagnosis and initiation appropriate therapy in individuals living with the disease.

Key words: Cluster of differentiation 4 count, human immunodeficiency virus status, North India, pneumocystosis

INTRODUCTION

Pneumocystis jirovecii pneumonia (PcP) is an opportunistic infection (OI) causing life-threatening pneumonia in immunocompromised patients and it still remains a common opportunistic disease in human immunodeficiency virus (HIV) infected individuals.[1] As compared to developed countries, the epidemiology and clinical spectrum of PcP have not been clearly defined and well documented in developing countries.[2] Hence, studies on the profile of pneumocystis infection in individuals with clinical suspicion of HIV in developing countries are needed to be carried out. This retrospective study was carried out in a tertiary care institution to study the occurrence of *P. jirovecii* infection in clinically suspected individuals and to study its association with HIV status and cluster of differentiation 4 (CD4) cell count of those patients.

MATERIALS AND METHODS

Our laboratory records were reviewed to collect 2 years data (January 2012 to December 2013) of

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respiratory sample analysis of clinically suspected pneumocystis infection patients with known/unknown HIV status and CD4 counts. During the study data inclusion period, both expectorated and induced sputum samples from clinically suspected pneumocystis infection were processed in our laboratory. Uniform sample processing and staining procedure were followed in all patients. The smears were prepared and fixed with acetone as per the manufacturer's instruction and were subjected to direct fluorescent antibody (DFA) stain using a commercially available kit (MERIFLUOR® Pneumocystis - Meridian Bioscience, Inc., Italy). The stained slides were screened using a fluorescent microscope with a ×10 followed by ×40 dry objective for the presence of trophozoites and cysts with typical apple green fluorescence. To assess reproducibility, two slides were made for each specimen and were examined separately. We collected the data regarding the HIV status and CD4 cell counts of the study group. The CD4 count was done only for HIV reactive individual in our institution.

RESULTS

Data of 45 eligible patients were analyzed in our study. The patients included in the study ranged from 2 to 60 years of age with male:female ratio of 31/14 (2.2:1). The mean age of the study cohort was 35.1 years. Most of the male patients (34/45; 75%) belonged to age groups of 21–50 years and the majority of the female patients (6/45; 13.3%) belonged to 21–30 years age group [Table 1]. Of all the patients, 26 (57.7%) were HIV reactive [7 out of 13 (53.8%)patients in 2012 and 19 out of 32 (59.3%) patients in 2013], whereas, 19 (42.2%) were of unknown HIV status. Among the HIV reactive individuals 69.2% (18/26) and among the unknown HIV status individuals 68.4% (13/19) were males. The result of CD4 count done for the HIV reactive individuals is shown in Table 2. Fourteen out of 26 (53.8%) had CD4 count of <200 cells/mm^3 whereas, 12 (46.2%) patients had CD4 count of >200 cells/mm^3.

Table 1: Patient demography of human immunodeficiency virus reactive and human immunodeficiency virus status unknown patients

Age	HIV reactive (*n*=26) *n* (%)		HIV unknown status (*n*=19) *n* (%)		Total (*n*=45) (%)
	Male	Female	Male	Female	
0-10	1 (3.8)	0 (0)	1 (5.2)	0 (0)	2 (4.4)
11-20	2 (7.6)	1 (3.8)	1 (5.2)	0 (0)	4 (8.8)
21-30	4 (15.3)	3 (11.53)	2 (10.5)	3 (15.7)	12 (26.6)
31-40	5 (19.2)	1 (3.8)	4 (21)	1 (5.2)	11 (24.4)
41-50	4 (15.3)	2 (7.6)	3 (15.7)	2 (10.5)	11 (24.4)
51-60	2 (7.6)	1 (3.8)	2 (10.5)	0 (0)	5 (11.1)

HIV=Human immunodeficiency virus

The result of DFA staining of the patients is shown in Table 3. 20 patients (8 in 2012 and 12 in 2013) were confirmed with pneumocystosis by DFA (15 male and 5 female) irrespective to their HIV status. Most of the males belonged to 21–40 years of age group, whereas most females were of the age group of 11–60 years. Among the pneumocystis positive individuals 9 were HIV reactive (4 in 2012 and 5 in 2013), whereas 11 were of unknown HIV status. Among the 14 HIV reactive individuals who had CD4 count of <200 cells/mm^3 4 were positive and 10 were negative for pneumocystosis with DFA staining. Among the 12 HIV reactive individuals who had CD4 count of >200 cells/mm^3 5 were positive and 7 were negative for pneumocystosis with DFA staining.

DISCUSSION

Opportunistic Infections are an important cause of morbidity and mortality among HIV-infected individuals. The use of highly active anti-retroviral therapy (HAART) and pneumocystis prophylaxis have dramatically reduced the number of cases of PcP. Moreover, pneumocystosis continues to occur among persons who are unaware of their HIV infection, and also in patients who fail to seek medical care, or adhere to or respond to anti-retro viral therapy (ART) or pneumocystis prophylaxis.[3]

World Health Organization (WHO) recommends starting of empiric therapy for PcP on the basis of its clinical case definition by a number of local guidelines which have been incorporated into the management protocols in resource-limited settings.[4] This approach may be acceptable in the correct clinical and epidemiological context, but inaccurate diagnosis carries a number of important implications in resource-poor countries.[5] Moreover, large and increasing numbers of patients with HIV-associated respiratory diseases has necessitated the use of rapid and accurate diagnostic tests for the identification of *P. jirovecii*. The DFA stain is a sensitive and convenient method for detection of *P. jirovecii*. It has the advantage of being simpler requiring less time and fewer reagents than indirect fluorescent-antibody, Gomori methenamine silver, and modified toluidine blue O stains.[6,7] Hence, we used DFA staining in our study.

Among the 45 patients with a clinical suspicion of pneumocystinosis included in our study, 19 (42.2%)

Table 2: Cluster of differentiation 4 count (cells/mm^3) of human immunodeficiency virus reactive individuals

	<50 (%)	51-100 (%)	101-150 (%)	151-200 (%)	201-250 (%)	>250 (%)
HIV reactive (*n*=26)	2 (7.6)	1 (3.8)	4 (15.3)	7 (26.9)	7 (26.9)	5 (19.2)

HIV=Human immunodeficiency virus

Table 3: CD4 count and direct fluorescent antibody staining profile of human immunodeficiency virus reactive and individuals with unknown human immunodeficiency virus status

DFA staining profile	HIV reactive (*n*=26)						Unknown HIV status (*n*=19)
	CD4 <50	CD4 51-100	CD4 101-150	CD4 151-200	CD4 201-250	CD4 >250	
DFA* positive (*n*=9) (%)	0	0	1 (11)	3 (33)	3 (33)	2 (22)	11 (57.8)
DFA* negative (*n*=17) (%)	2 (11.7)	1 (5.8)	3 (17.6)	4 (23.5)	4 (23.5)	3 (17.6)	8 (42.1)

*DFA=Direct fluorescent antibody staining; HIV=Human immunodeficiency virus

had unknown HIV status. This highlights the importance of unknown HIV status in patients with clinically suspected pneumocystis infection in our country. The mean age of HIV reactive state in our study was 35.1 years, which is similar to that reported in previous studies.[8-10] In contrast to male (21–50 years) female patients in our study were of slightly younger age group (21–30 years). This finding is similar to that reported previously.[8-12] This could be due to the general trend of having younger female partners in India. Another important observation in our study is that HIV infection still prevalent in patients in economically productive age group.

In our study, 20 out of 45 patients (44.4%) were positive for pneumocystinosis by DFA. Among the HIV reactive individuals, 34.6% (9 out of 26) of patients were positive for pneumocystinosis by DFA. In their study, Choe *et al*.[13] reported 55.3% HIV reactive patients to be positive for PcP by DFA. Earlier studies in the USA showed PcP as the most frequent cause of pulmonary infections (85%) and was also the first cause of hospital admission in HIV patients.[14] However, The reported incidence of PcP is between 32% and 45% in the current literature.[8,15-17]

Among the individuals diagnosed to have pneumocystinosis by DFA, 15/20 (75%) were male and 5/20 (25%) were female in our study. Most males belonged to 21–40 years of sexually active age group and the females belonged to age group of 11–60 years. In a study in South Africa, out of 41 patients, 77.7% (23 out of 27 females were positive for *P. jirovecii*, whereas, 50% (7 out of 14) males were positive for *P. jirovecii*. The females mostly belonged to 20–39 years of age who were older than in our study, whereas 5/7 (71.4%) males were of 20–39 years of age, which was similar to our study.[18]

The CD4 counts <200 cells/mm^3 is an important risk factor for OIs in HIV infection and CD4 count is considered the best short-term predictor of OIs.[8] Currently, The National Institutes of Health, the Centers for Disease Control and Prevention and the HIV Medicine Association of the Infectious Diseases Society of America guidelines mentions that prophylaxis against PcP may be discontinued safely in HIV-infected individuals when CD4 counts are maintained >200 cells/mm^3 for over 3 months on antiretroviral therapy.[19] Jensen *et al*.[20] and Masur *et al*.[21] in their respective studies have reported that around 95% of their patients with of PcP had a CD4 count of <250 cells/mm^3. However, in our study, 44.4% (4 out of 9) among the DFA positive individuals had a CD4 count <200 cells/mm^3 which indicates a poor correlation between CD4 count and DFA positivity in the diagnosis of pneumocystosis. The discrepancy between CD4 count and the DFA positive cases of pneumocystosis [Table 3] in our study could be due to the time difference between CD4 count increase and clearance of pneumocystis antigen from the body. We cannot comment the about the later because we did not have the data about the duration of HAART or pneumocystis prophylaxis received by the patients.

Nearly 58% (11 out of 19) individuals with unknown HIV status were DFA positive for pneumocystosis in our study. The CD4 count was not known in them because, as per National AIDS Control Organization (NACO) guidelines CD4 count being an expensive test is to be done only in HIV reactive individuals as a guide to therapy. Hence, HIV status unknown patients might had HIV infections or any other immunodeficiency state that predisposed them to PcP infection. Our finding emphasizes the need for increasing resources in a developing country like ours to diagnose HIV, other immune deficiency states in patients with pneumocystis infection.

The major limitation of our study is that our study is a retrospective one. Hence, data regarding the duration of HAART and/or pneumocystis prophylaxis was not available to us. Another limitation in our

study was that we did not have any data about the immune status and CD4 count of patients with unknown HIV status. Their actual HIV status and the CD4 count would help in knowing the actual PcP infection burden in the society and for an effective HAART therapy to be implemented to help in lessening the disease burden in the community.

CONCLUSION

Pneumocystis pneumonia is still prevalent in North India and is mainly affecting patients in economically productive and sexually active age group. To diagnose pneumocystosis, DFA remains an easily available method in resource-limited settings where the polymerase chain reaction assay cannot be developed and standardized to be followed routinely. As a rapid screening method, DFA can be used to detect *P. jirovecii* in respiratory specimens. Appreciating the actual HIV or immunodeficiency status and the CD4 profile of an individual with symptoms of pneumocystis infection will help the clinicians in early diagnosis and initiation appropriate therapy in individuals living with the disease.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. de Armas Rodríguez Y, Wissmann G, Müller AL, Pederiva MA, Brum MC, Brackmann RL, *et al. Pneumocystis jirovecii* pneumonia in developing countries. Parasite 2011;18:219-28.
2. Morris A, Lundgren JD, Masur H, Walzer PD, Hanson DL, Frederick T, *et al.* Current epidemiology of pneumocystis pneumonia. Emerg Infect Dis 2004;10:1713-20.
3. Morris AM, Swanson M, Ha H, Huang L. Geographic distribution of human immunodeficiency virus-associated *Pneumocystis carinii* pneumonia in San Francisco. Am J Respir Crit Care Med 2000;162:1622-6.
4. World Health Organization. WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children. Geneva: WHO Press; 2007.
5. Curtis JR, Yarnold PR, Schwartz DN, Weinstein RA, Bennett CL. Improvements in outcomes of acute respiratory failure for patients with human immunodeficiency virus-related *Pneumocystis carinii* pneumonia. Am J Respir Crit Care Med 2000;162(2 Pt 1):393-8.
6. Ng VL, Virani NA, Chaisson RE, Yajko DM, Sphar HT, Cabrian K, *et al.* Rapid detection of *Pneumocystis carinii* using a direct fluorescent monoclonal antibody stain. J Clin Microbiol 1990;28:2228-33.
7. Wolfson JS, Waldron MA, Sierra LS. Blinded comparison of a direct immunofluorescent monoclonal antibody staining method and a Giemsa staining method for identification of *Pneumocystis carinii* in induced sputum and bronchoalveolar lavage specimens of patients infected with human immunodeficiency virus. J Clin Microbiol 1990;28:2136-8.
8. Gautam H, Bhalla P, Saini S, Uppal B, Kaur R, Baveja CP, *et al.* Epidemiology of opportunistic infections and its correlation with CD4 T-lymphocyte counts and plasma viral load among HIV-positive patients at a tertiary care hospital in India. J Int Assoc Physicians AIDS Care (Chic) 2009;8:333-7.
9. Kothari K, Goyal S. Clinical profile of AIDS. J Assoc Physicians India 2001;49:435-8.
10. Spacek LA, Griswold M, Quinn TC, Moore RD. Total lymphocyte count and hemoglobin combined in an algorithm to initiate the use of highly active antiretroviral therapy in resource-limited settings. AIDS 2003;17:1311-7.
11. Usha Rani N, Reddy VV, Prem Kumar A, Vijay Kumar KV, Ravindra Babu G, Babu Rao D. Clinical profile of *Pneumocystis carinii* pneumonia in HIV infected persons. Indian J Tuberc 2000;47:93.
12. Wadhwa A, Kaur R, Agarwal SK, Jain S, Bhalla P. AIDS-related opportunistic mycoses seen in a tertiary care hospital in North India. Indian J Med Microbiol 2007;56:1101-6.
13. Choe PG, Kang YM, Kim G, Park WB, Park SW, Kim HB, *et al.* Diagnostic value of direct fluorescence antibody staining for detecting *Pneumocystis jirovecii* in expectorated sputum from patients with HIV infection. Med Mycol 2014;52:326-30.
14. Jung AC, Paauw DS. Diagnosing HIV-related disease: Using the CD4 count as a guide. J Gen Intern Med 1998;13:131-6.
15. Udwadia ZF, Doshi AV, Bhaduri AS. *Pneumocystis carinii* pneumonia in HIV infected patients from Mumbai. J Assoc Physicians India 2005;53:437-40.
16. Bava AJ, Cattaneo S, Bellegarde E. Diagnosis of pulmonary pneumocystosis by microscopy on wet mount preparations. Rev Inst Med Trop Sao Paulo 2002;44:279-82.
17. Costiniuk CT, Fergusson DA, Doucette S, Angel JB. Discontinuation of *Pneumocystis jirovecii* pneumonia prophylaxis with CD4 count, 200 cells/mL and virologic suppression: A systematic review. PLoS One 2011;6:e28570.
18. Govender S, Plessis SJ, Ocana GS, Chalkley LJ. Prevalence of *Pneumocystis jirovecii* and *Mycoplasma pneumoniae* in patients presenting with pneumonia at hospitals in Port Elizabeth. South Afr J Epidemiol Infect 2008;23:21-4.
19. Panizo MM, Reviákina V, Navas T, Casanova K, Sáez A, Guevara RN, *et al.* Pneumocystosis in Venezuelan patients: Epidemiology and diagnosis (2001-2006). Rev Iberoam Micol 2008;25:226-31.
20. Jensen BN, Gerstoft J, Højlyng N, Backer V, Paaske M, Gomme G, *et al.* Pulmonary pathogens in HIV-infected patients. Scand J Infect Dis 1990;22:413-20.
21. Masur H, Ognibene FP, Yarchoan R, Shelhamer JH, Baird BF, Travis W, *et al.* CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Ann Intern Med 1989;111:223-31.