ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 06, pp.4525-4527, June, 2019

DOI: https://doi.org/10.24941/ijcr.35539.06.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PNEUMOCYSTIS CARINII PNEUMONIA IN HIV INFECTED PATIENTS FROM SOUTH INDIA

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ARTICLE INFO

Article History:
Received 27th March, 2019
Received in revised form
13th April, 2019
Accepted 17th May, 2019
Published online 30th June, 2019

Key Words:
Pneumocystis Carinii Pneumonia,
HIV/ AIDS,
Opportunistic Infection.

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ABSTRACT

Pneumocystis carinii pneumonia (PCP) is the most common opportunistic infection in AIDS patients. To find out the prevalence of PCP among HIV infected patients, to elicit its association with pulmonary tuberculosis and gender, and to find out the superiority of the commonly used stains to detect pneumocystis. After getting approval from Institutional Ethical Committee, this study was carried out among 144 newly detected HIV infected patients who satisfied a presumptive diagnosis of PCP as per CDC/CDSC, attending Thoracic medicine department, Govt hospital. HIV infection was established by two different ELISA tests. Induced sputum was collected from each patient and processed for PCP by Giemsa and Toluidine blue staining, and for acid fast bacilli by Ziehl Neelsen method. The data were analyzed statistically. Among 144, PCP was identified both by Giemsa and Toluidine blue staining in 77. In addition, Giemsa helped to identify two 2 more cases of PCP. However, there was no statistical difference ($P < 0.53$), among the two stains used. Prevalence of PCP in HIV positive TB negative, HIV and TB co-infected group was 61%, and 54% respectively. The PCP among symptomatic HIV infected patients was 54.8%. The results of the study suggest that Giemsa staining is user-friendly.

Citation: Kuppamuthu Ramakrishnan, Uma A., Senathipathy, R., Vijayaraj, S., Ponniah Thirumalaikolundu Subramanian, Rathinasabapati, R and Rajesh Mondal.. **2019.** "Pneumocystis Carinii Pneumonia in HIV Infected Patients from south India", *International Journal of Current Research*, 11, (06), 4525-4527.

INTRODUCTION

Pneumocystis carinii is a protozoan parasite, discovered in 1909 by Charles Chagas, and it has long been recognized in patients with impaired immunity. It was renamed as *Pneumocystis jiroveci,* since 1999 (Frenkel, 1999). *Pneumocystis carinii* pneumonia (PCP) is the most common opportunistic infection in acquired immuno-deficiency syndrome (AIDS) patients (Bryan et al., 2008) from the developing world and elsewhere. Immunosuppression especially involving T-cell function leads to infection resulting in disease. In the early stage of PCP, the chest radiograph shows fine bilateral perihilar diffuse infiltrate, which progress to the interstitial alveolar butterfly pattern (Usha, 2000). Early accurate diagnosis of this opportunistic infection (OI) helps in effective disease management. The present study was undertaken to find out the prevalence of *pneumocystis carinii* pneumonia among HIV infected patients at Madurai, Southern India, and to elicit its association with pulmonary tuberculosis and gender, and to find out the superiority of the commonly used stains to detect pneumocystis.

MATERIALS AND METHODS

This prospective study was carried out among the newly detected HIV infected patients with respiratory manifestations attending the department of Thoracic medicine, Government Rajaji Hospital, Madurai, over a two years period from January 2008 to December 2010. HIV infection was established by two different ELISA tests *i.e., Innotest* ™ HIV $_1$/HIV$_2$ (Belgium) and Lab systems (Finland). The presumptive diagnosis of PCP was made in a HIV infected person as per CDC/CDSC, guidelines (4, 5) (Centre for Disease Control/Communicable Disease Surveillance Centre) which are furnished below

- Dyspnoea on exertion/non-productive cough of recent onset,
- Chest X-ray showing diffuse bilateral interstitial infiltrates.
- Arterial hypoxaemia,
- No evidence of bacterial pneumonia.

All patients selected for the study were motivated and trained to collect induced sputum according to the procedure of

(Bigby, 1986). Sputum induction was carried out with progressive concentration of hypertonic saline solution (5% of 10ml) delivered through a nebuliser ("MEDEL" India LTD).

Exclusion criteria: Patients with malignancy, diabetes mellitus, and other end organ disorders were excluded clinically and by laboratory means. Pregnant women, patients on immunosuppressives or any other antimicrobials including cotrimoxazole or suffering from other serious illnesses and history of present or past tobacco smoking were excluded. The study was carried out after getting an approval from Institutional Ethical Committee. Informed consent was obtained before HIV testing and for participation in the study. Counselling was provided to all patients. Processing of specimens for Pneumocystis carinii pneumonia. Induced sputum was collected from each patient and processed for evaluation. Equal amount of the specimen and DTT (Dithiotheritol) were mixed (vortexed), and kept at room temperature for 30 minutes.

The specimen was then centrifuged at 2500 rpm for 15 minutes. Smear was made from the deposit in two new glass slides separately. Giemsa Staining: One smear was fixed with methanol and then stained with Giemsa for half an hour. Slide was washed under running tap water and examined under oil immersion. Toluidine Blue staining: Another smear was fixed with freshly prepared sulfation reagent (4.5% ml Acetic acid and 1.5% Sulphuric acid) and washed with tap water. Later the slide was treated with toluidine blue for 3 minutes. Then the slide was dipped thrice in increasing concentration of alcohol (70%, 85% and absolute alcohol) and finally dipped in xylol. Slide was air dried and mounted, and observed under oil immersion microscope for PCP. Ziehl-Neelsen's staining: The sputum samples were also subjected to Ziehl-Neelsen's staining for the diagnosis of TB and the CD4 count was carried out using Becton Dickinson flowcytometer (USA). Statistical analysis: Statistical analysis was carried out using SPSS 15 Chicago version Chi-square test was performed to find out the superiority of two stains (Giemsa and Toluidine blue) for identifying PCP among patients, and to elicit gender difference, as well as for the occurrence of PCP among TB positive and TB negative patients.

RESULTS

A total of 144 HIV patients who satisfied the selection criteria were included. Among them there were 84 males and 60 females, and their age ranged from 21 to 55 with a median and mean of 33 and 33.2 years respectively. Based on the positive status for TB, the total cases were classified into HIV positive TB negative and HIV positive TB positive. Accordingly the analysis was made. Among the 144 cases subjected to Giemsa staining and Toluidine blue staining, PCP was identified by both stains in 77. Giemsa helped to identify two more cases which could not be identified by Toluidine blue. There was no statistical difference ($P < 0.53$), among the two stains used in this study. PCP was positive in 46 males and 33 females. Prevalence of *Pneumocystis carinii* pneumonia in HIV positive TB negative, and HIV and TB co-infected group was 61%, and 54% respectively. Acid fast bacilli was identified in sputum of 74 (51.4%) and negative in the rest. PCP infection among HIV infected individuals was independent of their positive status for pulmonary tuberculosis and gender. The CD4 count among them varied from 95 to 198 with median of 157cells/cumm.

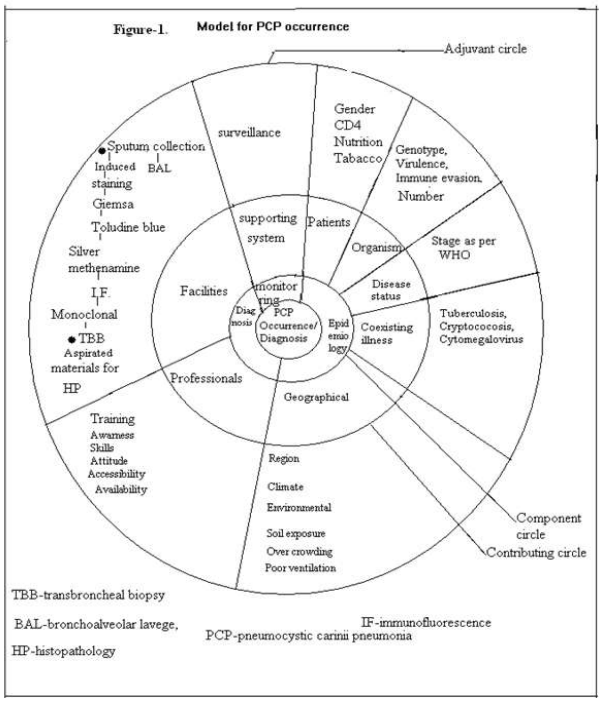

Figure 1. Pictorial model

DISCUSSION

HIV infected patients are susceptible to lower airway infections by various agents, and the occurrence is influenced by the interaction between the agent and host. Two respiratory infections with high prevalence even in the era of HAART are PCP and pulmonary tuberculosis**.** The greatest risk for PCP occurs, when the CD4+ count falls below 200 cells/cumm (Singhal, 2005) as seen in this study. To diagnose PCP, induced sputum can be used as an alternative to invasive and expensive bronchoalveolar lavage (BAL) (Usha, 2000; Pitchenik, 1986). PCP in India: In the Indian context, PCP was first reported in 1993 (Singh, 1993). Arora (10) from New Delhi observed PCP to be the second most common opportunistic infection among HIV patients. It was also noticed with other pulmonary infections, including TB, cryptococcosis, cytomegalovirus etc., (Deshmukh, 2003). In the present study PCP was identified from (79) 54.8% of established HIV/AIDS cases with and without TB alone. The prevalence of PCP in Indian series varied from 6.1% to 60% (Kumarasamy, 2003; Rajeshwari, 2007) and observed more among males. In the present study there was no gender difference. Global status of PCP: Prevalence rate of PCP in other countries varied from 3 to 74%. (Koltz et al., 2007 ; Armand, 2001).

Reasons for variations in the prevalence: Three major components such as epidemiological, diagnostic and monitoring aspects inter play with each other for the occurrence and / or diagnosis of PCP among HIV positive individuals and explain for the variations in different studies.
The influencing factors under epidemiology are factors related to patients, organism, disease status, co-existing illness and geographical aspects.Ambient air pollution have been suggested to be as a risk factor of PCP (Alvaro-Meca et al., 2015). With reference to diagnosis two aspects have to be considered and they are factors related to professional and facilities. In addition, monitoring aspects of HIV infected patients with committed supervisory system help to identify PCP in larger numbers. The details and other adjuvant factors

for PCP are shown in a pictorial model (figure 1). In Indian series, Giemsa stain was used mostly and with that the prevalence of PCP among HIV varied from 12.5 to 32% (Usha, 2000; Udwadia et al., 2005). The other stains used were Toluidine blue, Gomori methenamine silver (Singh, 1993), Immunofluorescence (Udwadia, 2005), and Monoclonal antibody. Giemsa stain is convenient, economical, user friendly, stains nucleus quickly and feasible at all levels. Microscope in PCP: Microscope constitutes the mainstay of diagnosis and involves visualization of trophozoites or cyst forms (Singhal, 2005). The sensitivity and specificity for Giemsa and Toluidine blue staining ranging from 50-84 and 49-60 percent (Naimey, 1995) respectively. Immunofluorescence staining of aspirates is good but it is very expensive method for diagnosis and facilities are not available in all places, in addition to adds to the financial burden (Mathews, 2000). It is likely that the use of more than one staining technique may increase the diagnostic yield and help to identify the cases which are likely to be missed by the other stain. In the present study, there was no statistical difference among the two stains. Giemsa and toluidine blue may be used for routine diagnostic purposes in resource limited settings. Strength: The strength of the present study was rigid criteria adopted for selection of all cases and standard methods followed for sputum collection as well as processing the samples by senior microbiologist. This study has shown that induced sputum can be done as an out patient procedure even in a busy hospital setting, as it does not require any special equipment. Among the stains used (Giemsa and Toluidine blue) in this study, Giemsa stain is simple and results available quickly, so that appropriate treatment/chemoprophylaxis may be initiated promptly.

Acknowledgements: The help offered by Dr. S. Soumyaswaminathan, Former Director, NIRT/ICMR, for critical review of this manuscript. Dr. S.C. Vivekanandan, Government Rajaji Hospital, Ms. Janaki M.Sc., student, Mr. Kannan, Health Visitor, Revised National TB Control Programme, Madurai for sample collection and Mr. Balasubramanian, Former Statistician (Centre for Research in Medical Entomology / ICMR) Madurai, Tamilnadu are gratefully acknowledged.

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