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Detection of *Pneumocystis carinii (jiroveci)* from Iraqi Patients with Lower Respiratory Tract Infections

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Abstract

Background *Pneumocystis carinii* is one of the rare fungi which cause pneumonia in immunocompromised patients. It is important to detect the fungus from the clinical specimens of suspected patients by laboratory tests.

Objective To identify *Pneumocystis carinii* from immunocompetent and immunocompromised patients with lower respiratory tract infections.

Methods This study included 300 patients suffering from lower respiratory tract infections of both immunocompetent (150) and immunocompromised (150) patients attending the Teaching Hospital in Mosul/Iraq. The clinical specimens collected were samples of sputum (247), and bronchial wash (80). The identification of *Pneumocystis* staining methods.

Result The organism was detected from 8 immunocompromised patients with pneumonia. Seven out of the 8 patients had carcinoma.

Conclusion *Pneumocystis carinii* is an opportunistic fungus which is an important pathogen in immunocompromised patients.

Key words *Pneumocystis carinii (jiroveci),* pneumocystis pneumonia, respiratory tract infection.

List of abbreviation: LRT = lower respiratory tract, AFB = acid fast bacilli, PCP = pneumocystis carinii pneumonia, HIV = human immunodeficiency virus, AIDS = acquired immunodeficiency syndrome

Introduction

Pneumocystis carinii was originally thought to be a protozoan when first described in the early 1900, but the advent of molecular techniques has now firmly established *P. carinii* as a member of the fungal kingdom [1]. The name *P. jiroveci*, to distinguish the organism found in human from physiological variants of *pneumocystis* found in other animals, was first proposed in 1976, in honor of Ottojiroves [2].

The occurrence of *Pneumocystis carinii* is worldwide, except in Antarctic, and is commonly found in the lungs of healthy individuals [3]. Most children are believed to have been exposed to the organism by age 3-4 years [4,5]. The organism causes pneumocystis pneumonia [2]. It affects only people with weakened immune system, especially people who are human immunodeficiency virus (HIV) positive [6]. The use of combination immunosuppressive agents is associated with reports of *P. jiroveci* pneumonia [7]. Infection occurs following the inhalation of spores, or by the reactivation of a latent infection [8].

The disease form when defects exist in both cellular and humoral immunity [5]. Once inhaled, the trophic form of the organism attaches to the alveoli and starts replication, then gradually fills the alveoli [9]. The organism is found in three distinct morphological stages. The trophozoite or trophic form, the sporozoite which is a precystic form and the cyst, which contain several intracystic bodies (2-8) or spores [5,10].

The aim of the present study is to identify *Pneumocystis carinii* from immunocompetent and immunocompromised patients with lower respiratory tract infection, using direct detection procedures namely different stains, and fluorescent microscopy.

Methods

This prospective study was conducted from April 2007 to June 2008 on 300 patients suffering from lower respiratory tract infections. Males were 175 (58.3%) and females were 125 (41.7%). Patient's age ranged from 1 to 89 (55.44 ± 17.9) years. The subjects included were of equal number, 150 apparently immunocompetent and 150 presumably immunocompromised patients.

Immunocompromised status was suspected in patients with different types of carcinoma and leukemia (46.0%), uncontrolled diabetes mellitus of > 5 years duration (25.3%), old tuberculous patients with negative acid fast bacilli (AFB) (10.7%), and chronic diseases under long-term corticosteroids therapy (18.0%).

Studied samples

A total of 327 specimens were collected from patients in the Ibn Sina Teaching Hospital (Respiratory Care Unit, Bronchoscopy Unit/ Wards) and from the Oncology and Nuclear Medicine Hospital, Mosul, Iraq.

The samples consisted of 227 sputum and 80 bronchial wash (27 patients with both sputum and bronchial wash).

The sputum of each patient was shaken by a vortex for 3-5 minutes, and the bronchial wash was centrifuged for 5 minutes, then the sediment was used for direct microscopical examination [11].

Pneumocystis carinii was identified by direct microscopical examination with different stains. Three slides were prepared from each specimen. One, wet mounted slide with 20% KOH solution and calcofluor stain (Becton Dickinson, USA), then examined under fluorescent microscope to detect the cysts. The other two fixed smears stained with Giemsa and Toludine blue stain to detect trophozoites and intracystic bodies by Giemsa stain and cysts by Toludine blue stain under 100x magnification of light microscope [6].

All the participants had given consent to participation in the research work which approved by Department of Microbiology / University Research in 21/6/2007 (4S/1329) and Teaching Hospitals (confirmed by the Center of Continuous Medical Education, No. 8021 in 2/7/2007).

Results

The patients were categorized according to the clinical entities. The most frequent clinical entity was pneumonia (49.6%). *Pneumocystis carinii* was identified in 8 cases with pneumonia. The organism was detected in bronchial wash and/or sputum of immunocompromised patients only. The patients were 6 males and 2 females, four of them were farmers, and 7 had malignancies under radiation and/or cytotoxic therapy (Table 1).

The wet prepared slide showed the cysts under 40X lens of fluorescent microscope (Figure A). Stained smear with Giemsa stain showed the intracystic bodies and trophozoites (Figure B and C). The third slide was stained with toludine blue stain for the appearance of the cysts also (Figure D). The organism was identified from 5 cases singly (without other fungus), and in the other 3 cases were mixed with yeast species. However, in 7/8 of these cases, heavy growth of bacteria appeared when the specimens inoculated on blood and MacConkey's agars.

Discussion

Pneumocystis carinii is a rare cause of infection among the general population, but is a major pulmonary pathogen for the immunocompromised patients mainly those with acquired immunodeficiency syndrome (AIDS) and malignant diseases [12,13]. The organisms were detected in immunocompromised patients from cases of pneumonia. The main predisposing factor for these patients was malignancy, which was diagnosed in 7 out of the 8 patients (Table). A previous study mentioned that *Pneumocystis*

carinii pneumonia (PCP) is a common opportunistic infection in patients with lymphoma and leukemia [14]. In a more recent report, the immunocompromised patients with no AIDS and at risk for PCP include individuals with hematological malignancies [15]. Furthermore, opportunistic organisms including *P. carinii* caused experimental infection in immunosuppressed mice [16].

Table 1. Clinical data of the patients with *Pneumocystis carinii*.

N	Sex	Age (yr)	Occupation	Predisposing factors	specimen examined	Main symptoms & signs
1	♂	80	farmer	carcinoma (cytotoxic therapy)	sputum & bronchial wash	Cough (8/8) Dyspnia (7/8) Fever (6/8) Haemoptysis (5/8)
2	♀	65	farmer	bronchial carcinoma	bronchial wash	
3	♂	35	unemployed	lymphoma (cytotoxic & RT)	sputum	
4	♂	60	farmer	asthma (steroid therapy)	sputum	
5	♂	26	unemployed	leukemia (cytotoxic therapy)	sputum	
6	♀	42	housewife	leukemia (cytotoxic therapy)	sputum	
7	♂	49	worker	leukemia (cytotoxic therapy)	sputum	
8	♂	65	farmer	leukemia (cytotoxic therapy)	sputum	

RT = Radiotherapy

The organism was identified from bronchial wash and/or productive sputum of 8 patients out of the 300 cases studied. A reported study showed that 24 (11.8%) of 204 clinical specimens (bronchial aspirate, induced sputum) were positive for *P. carinii* [17]. During the study, the productive sputum was examined, not the induced type because this study not only for detection of *P. carinii*, but for the isolation of other fungi in the lower respiratory tract (LRT), and may be affected by the hypertonic saline used for sputum induction.

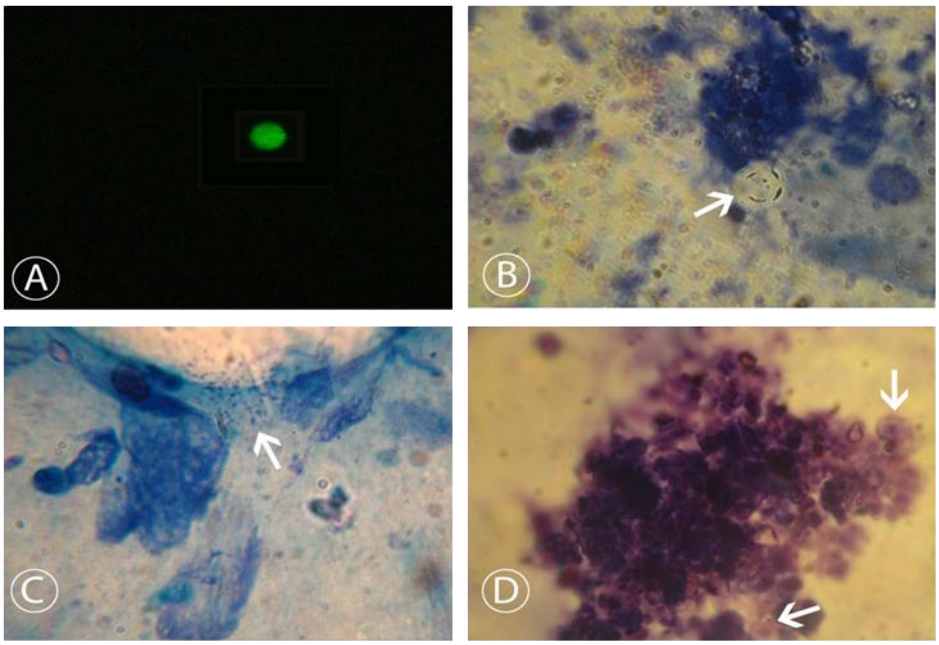

Fig. Sputum & bronchial wash showing *Pneumocystis carinii* (A) 20% KOH and calcofluor stain (spherical cyst; cyst wall and thickening intensely flurescent) 40X. (B) Giemsa stained smear (4 purple intracystic bodies -arrow) 100X. (C) Giemsa stained smear (small extracellular blue trophozoites - arrow) 100X. (D) Toludine blue stained smear (many spherical violet cysts - arrow) 100X.

The symptoms and signs of PCP are non-specific because the infection occurs in debilitated patients with other primary diseases [18]. However, the diagnosis of such cases was

difficult. The identification of the organisms during the study depended on the direct examination of each clinical specimen by different stains, because *P. carinii* cannot be cultured [19]. Previous studies reported that calcofluor white is a fungal cyst-wall stain [6]. Furthermore, toludine blue stain also allows diagnosis of *P. carinii* cysts [20], and trophic forms can be detected with Giemsa stain [6].

The isolation of concomitant bacteria and yeast from the sputa of most pneumocystic cases was also reported by other investigators [21]. Large amount of sputum was produced by patients who had *P. carinii* mixed with positive bacterial cultures. Such findings had been also reported by others [5,6].

In conclusion, *Pneumocystis carinii* was detected in immunocompromised patients with lower respiratory tract infections by direct microscopic examination of the clinical specimens with different staining methods.

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Author contributions

Dr. Manahil PhD student write the manuscript and Prof. Zainalabideen supervised the research work.

Declaration of interest

The authors declare no conflict of interest.

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