PREVALENCE OF PNEUMOCYSTIS JIROVECI AMONG PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

Thesis

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Contents

Abbreviations

- AIDS : Acquired immunodeficiency syndrome
- ALL : Acute lymphoblastic leukemia
- BAL : Bronchoalveolar lavage
- BDG : β-D-Glucan
- CD : Cluster of differentiation
- CT : Computed tomography
- CW : Calcofluor white
- CF : Complement fixation
- CIE : Counter-immunelectrophoresis
- CBC : Complete blood count
- DTT : Dithiothreitol
- DS : Dihydropteroate synthase
- DHPS : Dihydrofolate synthetase
- DNA : Deoxyribonucleic acid
- DQ : Diff-quick
- ESR : Erythrocyte sedimentation rate
- ELISA : Enzyme-linked immunosorbent assay
- EDTA : Ethylenediamine tetraacetic acid
- f.sp : Formae speciales
- GSG : Glucan synthetase gene
- GMS : Grocott-Gomori methenamine silver
- G6PD : Glucose-6 phosphate dehydrogenase
- gp : Glycoprotein
- HIV : Human immunodeficiency virus
- HAART : Highly active antiretroviral therapy
- HCL : Hydrochloric acid

- ITS : Internal transcribed spacer
- IL : Interleukin
- IgG : Immunoglobulin G
- IFN-γ : Interferon gamma
- IS : Induced sputum
- IFA : Indirect immunoflourescent antibody
- IF : Immunoflourescent
- IV : Intravenous
- KCl : Potassium chloride
- mtLSU rRNA : Mitochondrial large subunit of rRNA
- Msg : Major surface Glycoprotein
- mRNA : Messenger ribonucleic acid
- MSN : Methenamine silver nitrate
- mtSSU rRNA :Mitochondrial small subunit of rRNA
- mM : Millimolar
- $MgCl_2$: Magnesium chloride
- µl : Microliter
- ng : Nanogram
- PFGE : Pulsed-field gel electrophoresis
- P. carinii : Pneumocystis carinii
- P. jiroveci : Pneumocystis jiroveci
- PCP : Pneumocystis pneumonia
- PCR : Polymerase chain reaction
- PBS : Phosphate buffer saline
- RNA : Ribonucleic acid
- RT–PCR : Reverse transcriptase polymerase chain reaction
- rRNA : Ribosomal ribonucleic acid
- SIDS : Sudden infant death syndrome
- SCID : Severe combined immunodiffeciency

- SS : Single strength
- s-LDH : Serum lactate dehydrogenase
- SMX : Sulphamethoxazole
- TEM : Transmission electron microscopy
- TNF : Tumor necrosis factor
- TNF-α : Tumor necrosis factor alpha
- TBO : Toulidine blue O
- TMP- SMX : Trimethoprim and sulphamethoxazole
- TMP : Trimethoprim
- TBE : Tris base, boric acid, EDTA
- UCS : Upstream conserved sequence
- UV : Ultraviolet

List of Tables

List of Figures

Page

List of Photos

INTRODUCTION:

Pneumocystis (*P.*) *jiroveci* previously named *Pneumocystis carinii* is an opportunistic eukaryotic pathogen causing life-threatening pneumonia in immunosuppressed patients ***(Linssen et al., 2006)***. Since its discovery in the early 1900s, it was thought to be a protozoan. Then in the 1980s, DNA analysis showed that this organism is, in fact, a fungal species ***(Sing et al., 2000)***.

The incidence of Pneumocystis pneumonia (PCP) has increased dramatically with the epidemic of human immunodeficiency virus (HIV) infection. Patients who are immunocompromized for reasons other than HIV infection are also at risk for pneumonia caused by *P. jiroveci* ***(Siripattanapipong et al., 2005)***. For non-HIV patients with PCP, predisposing factors include congenital immunodeficiency syndromes, administration of corticosteroids or cytotoxic agents in hematological malignancies, bone marrow or organ transplantations ***(Wong et al., 2007 and Hal et al., 2009).***

Since untreated PCP is associated with a high morbidity and mortality, especially in HIV-negative patients, a rapid and reliable diagnosis is mandatory ***(Linssen et al., 2006)***.

Diagnosis of PCP in the laboratory was, until few years ago, dependent on visualization of Pneumocystis organism in stained preparations of appropriate respiratory specimens using the Giemsa, Gomori methenamine silver stain (GMS), toluidine blue O stain, and fluorescein-conjugated monoclonal antibody [direct fluorescent-antibody (DFA) stain] ***(Su et al., 2007)***.

However, direct demonstration of the organisms (*P. carinii*) is often difficult or not possible because the trophozoites or cyst of the organism are either not present in abundant numbers or not uniformly spread out in the alveoli of infected lungs. In order to obtain sufficient organisms, this has required invasive samples from the alveoli, originally transbronchial biopsy samples, and subsequently broncho-alveolar lavage (BAL) fluid ***(Wakefiled, 2002).*** Moreover, microscopy is cumbersome and requires trained microscopists. Therefore, a rapid diagnostic technique which can identify the presence of a low number of organism is needed ***(Linssen et al., 2006).***

Molecular techniques have been developed for the detection of Pneumocystis, based on the identification of *P. jiroveci* DNA using the polymerase chain reaction (PCR). The most widely used locus for detection of *P. jiroveci* DNA is the gene encoding the mitochondrial large subunit rRNA (mtLSU rRNA), which has been shown to be a sensitive and specific mean of detection using PCR ***(Wakefield et al., 1990; Tamburrini et al., 1993).***

Furthermore, nested-PCR approach offered even a more sensitive and specific tool than the widely used single-PCR method for detecting *P. jiroveci* DNA and has been successfully applied to upper respiratory samples such as oropharyngeal and nasopharyngeal aspirates ***(Su et al., 2007)***.

AIM OF WORK:

The aim of the present study is to determine the prevalence of *Pneumocystis jiroveci* among non- HIV, immunocompromized patients suffering from various hematological malignancies.

THE GENUS PNEUMOCYSTIS

I-HISTORICAL OVERVIEW:

The history of the Pneumocystis genus began in Brazil, where **Chagas (1909)** first discovered Pneumocystis cystic forms in the lungs of guinea pigs inoculated with the blood of two children with trypanosomiasis **(Denis et al., 2008)**.

In **1910**, **Carini** found similar structures in rat lungs infected by the trypanosome group. The organism was mistaken for a demonstrated component of the trypanosome life cycle until **Delanoe and Delanoe (1912)** found that it was a separate organism and they suggested naming it *Pneumocystis (P.) carinii*: ‘‘Pneumo-’’ in relation to lung tropism, ‘‘cystis’’ because of its characteristic shape and ‘‘carinii’’ to honour Dr. Antonio Carini **(Kim, 2002).**

During searching for anti-*P. carinii* drugs, it was established that the *P. carinii* pneumonia did not respond to the broad-spectrum antifungal drug amphotericin B but it could be successfully treated with drugs that were active against protozoa. This finding contributed to the idea that *P. carinii* may be a protozoan. A protozoan identity also seemed more consistent with the failure of *P. carinii* to grow continuously in culture **(Stringer, 1996).**

However, in 1989, **Edman and his colleagues,** in USA, identified the sequence of the *P. carinii* gene encoding the RNA in the small ribosomal subunit (*16s-like rRNA* gene). The authors found that the *P. carinii 16s-like rRNA* gene sequence is much more similar to fungal sequences. Moreover, several protein-encoding genes have been isolated from rat derived *P. carinii*, the most interesting being the gene encoding elongation factor 3, a protein that has been found only in fungi so far.

Thus, *P. carinii* was placed on its own branch between the Ascomycetes and the Basidiomycetes but clearly within the fungal kingdom **(Stringer, 1996).**

The organism has been subsequently identified in a variety of mammalian host species including humans, laboratory animals, wild and zoo animals, and domesticated animals. The organisms identified in these species appeared morphologically similar and were thought to be the same organism. Thus, for most of the 20th century, *P. carinii* was thought of as a single organism capable of causing disease in a variety of mammalian hosts **(Laakkonen, 1997).**

Later on, the application of molecular genetic analysis to the study of Pneumocystis has confirmed the heterogeneity of the organism. Thus, the name *Pneumocystis jiroveci* became the official name for human Pneumocystis. This name was first proposed by Frenkel in 1976, in honor to the pathologist Jirovec who first reported the organism in humans. On the other hand, *P. carinii* refers to the original rat Pneumocystis **(Sandubete et al., 2002; Lu and Lee, 2008).**

II-TAXONOMIC CLASSIFICATION:

Pneumocystis is considered to have an intermediate and isolated position between Basidiomycota and Ascomycota. Most accurately the genus Pneumocystis is classified together with Schizosaccharomyces in the phylum Ascomycota, class Archiascomycetes, order Pneumocystidales in the family Pneumocystidaceae (Table 1) ***(Guarro et al., 1999).***

Table (1): Taxonomic classification of the Pneumocystis genus.

Phylmu	Class	Order	Family	Genus
Ascomycota	Archiascomycetes	**Shizosaccharomycetales**	**Shizosaccharomycetaceae**	**Shizosaccharomyces**
		Taphrinales	Taphrinaceae	Taphrina
				Lalaria
		Protomycetales	Protomyceteae	Protomyces
		Pneumocytidales	Pneumocytidaceae	Pneumocytis
	Euascomyctes			Endomyces
				Oosporidium
	Hemiascomycetes	Saccharomycetales	Ascoideceae	Ascoidea
			Cephaloascaceae	Cephaloascus
			Candidaceae	Candida
			Saccharomycetaceae	Saccharomyces
			Saccharomycodaceae	Saccharomycodes
			Endomyceteaceae	Endomyces
			Lipomycetaceae	Lipomycetaces

(Krutzman and Fell, 1998)

III-THE PNEUMOCYSTIS SPECIES:

Molecular genetic analyses made it clear that *P. jiroveci* organisms isolated from patients are quite different from animal-derived organisms and that Pneumocystis organisms from different animals are also different. These molecular data fit with previous demonstrations that