Original Article: Monitoring of Opportunistic Fungal Infections in Cases with PJP Symptoms Enduring Kidney Failure Diseases

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ABSTRACT

Fungal infections of respiratory system are the most common reason of death among immunosuppressed patients. Pulmonary candidiasis occurs as a primary bronchopneumonia or secondary type through blood diffusion. Primary bronchopneumonia of *Candida*, can also occur in patients which had been severely weakened due to tumors, neutropenic patients receiving extensive chemotherapy and infants with very low weight. Aspiration of infectious oral discharges in bronchi and their expansion in pulmonary parenchyma is the main pathway of transmission in *Candida* bronchopneumonia. In the current research, the most common fungi isolated from respiratory systems were *Candida* species (91.9%). We certified strains from 38 yeast species, which included almost all medically important yeasts, were cultured on the chromogenic medium and resulting colorful colonies were evaluated. The morphologies of the colonies on cornmeal agar considered as a primary guide for our identifications so then, four *Candida* species plus *C. neopforman* as shown at table 2 are the probable alternatives diagnosed in cases suspected to PJP. The results obtained in this work for yeast abundance complied with the findings of other researchers around the world, and indicated that there were almost similar patterns for yeast outbreak in different populations.

Introduction

Photocatalysts are semiconductors activated by the absorption of photons. If the energy difference of the layers is provided, the electron jumps from the capacitance band to their conduction band, which uses light energy for this excitation. Unlike metals, semiconductors, which have an attached environment of electrons, are solids with electrical conductivity between conductive

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and non-conductive materials. Semiconductors have two separate energy bands. In each band, the distance between energy levels is minimal, and in practice, a continuous spectrum of energy is seen. The distance between the conduction energy band and the capacitance is called the gap band, which contains the energy levels at which the electron cannot stay. The reason for using semiconductors as photocatalysts is due to their suitable electronic structure, light absorption, and charge transfer properties, and their long life in the excited state. Metal oxide semiconductors are the most suitable photocatalysts with optical abrasion resistance, such as titanium oxide (TiO_2), zinc oxide (ZnO), zinc sulfide (ZnS), iron oxide III (Fe_2O_3), cadmium sulfide (CdS) and zirconium dioxide (ZnO_2) [1].

Fungi cause some types of respiratory diseases in human by using several mechanisms; including colonization on epithelium layer, frequently exposure of lower respiratory tract with fungal contaminating spores, aspiration of oral normal flora into upper respiratory tract, invasion into endothelium by fungi hyphae and pseudohyphae(1). In some cases pulmonary fungal infection could be disseminated to reticuloendothelial system or other organs including bones, skin, central nervous system (CNS) and kidney. Major causing agents of respiratory tract infections include *Candida* species, *Pneumocystis jirovecii*, *Rhizopus arrhizus*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and some other hyalohyphomycetes. Hospital acquired fungal infections are routinely started by the contamination of air, medical devices, drug and solution bottles, over using of medical invasive procedures such as catheters and urethral sounds, ventilation systems and exposure to hospital cast members and visitors(2).

During recent decades, respiratory tract infections caused by opportunistic fungi have been increasing along with the frequency of high risk patients including immunosuppressions, corticosteroid therapies, invasive medical procedures, malignancies, chemotherapy and hospital long stay (3, 4).

There is a reverse correlation between the frequency of candida infections and the immunity competence(5). *Aspergillus* species are the second major cause of hospital fungal infections in immune compromised cases and followed by organisms including Mucorales, *Fusarium* species and other low frequent molds which are active only in immune suppressed patients (6, 7). One of the most important procedures for the diagnosis of respiratory fungal infections is isolation of fungal agents from clinical specimens and microscopic identification, although that can be embraced a diagnostic spectrum including normal colonization to an invasive situation(8). A fungal colonization could be started by a long stay of patients at hospital, medications and using of invasive medical procedures (3, 9, 10). A hospital fungal infection could be confirmed by microscopic investigation, frequently isolation on culture media, serologic and molecular detections (11, 12).

Considering importance of opportunistic fungi as the potent causes of hospital acquired infections, we studied and evaluated presence of the high frequent fungi in clinical specimens of cases with Pneumocystic jirovecci pneumonia (13, 23).

Materials and methods

In the present descriptive study, 200 cases with the symptoms and signs of lower respiratory tract (applied for PJP laboratory examinations), investigated for the opportunistic fungal infections or colonization. The age range included between 2 to 96 [24-27]. All of the cases were included long stay patients enduring immunosuppression for kidney transplantation and other high risk groups such as haematologic malignancies and chemotherapy users. Our subjects were respiratory tract specimens including bronchoalveolar lavage and sputums collected and transported to medical mycology center, Taleghani training hospital, UMS University, Urmia, Iran, for the PJP checking. The demographic information fulfilled by healthcare workers was consigned with the clinical specimens. The specimens were processed into 0.5 % pancreatin followed by micro-centrifuge

sedimentation at 5000 RPM, making wet KOH 10 % preparations and staining with giemsa and Grocott methenamine silver (GMS) kits [28-30].

An adequate amount of the sediments profiled into mycological basic culture, sabouraud dextrose agar (SDA) 4 % and incubated at 25-30°C for at least 48 hours. For the identification of isolated yeasts (Candida for example), a tip of colonies was sub cultured on CHROMagar Candida and cornmeal agar differential media. Macroscopic and microscopic features of fungal growth on differential media were considered, and used for the identification at the level of species by their carbohydrate assimilation patterns [31-33].

Results

Totally 200 respiratory tract specimens were collected for the laboratory checking of PJP and other opportunistic pulmonary infections. Direct and culture examinations showed 74 (37 %) cases with one or more fungal colonisation.

The cases with a fungal infection or colonization included 42 (56.7 %) male and 32 (43.2 %) female; they ranged between 2 to 96 years old.

As we showed at the table 1, Candida yeasts are the most frequent among all fungi; 68 cases (91.9 %), followed by *Aspergillus* and *Acremonium* species, *Cryptococcus neoformans* and Mucoral fungi. Invasive Candida elements were detected in 37 (50 %) cases.

Table 1: Primary detection of fungal elements in the respiratory tract specimens

Microscopic features	Sputum	BAL	Frequency (%)
***Candidia* invasive form**	04	33	37 (50)
***Candida* colonization**	05	26	31 (41.9)
***Aspergillus* (group flavi and fumigatus).**	-	03	03 (4)
***Acremonium* spp.**	-	01	01 (1.3)
***Cryptococcus* (encapsulate yeast)**	-	01	01 (1.3)
***Mucor* spp.**	-	01	01 (1.3)
total	09	65	74 (100)

All Candida cases were confirmed by the differential medium, CHROMagar Candida. According to table 2 data, *Candida albicans* and *Candida dubliniensis* were the most frequent (55.2 % and 28.9 %) respectively. Some other pathogenic yeasts such as *Cryptococcus neoformans*, *Candida glabrata* and *Candida tropicalis* were also identified using a dalamao Test on cornmeal agar (Medically important fungi, 2003).

Table 2: Comparative frequency of the yeasts isolated from BAL and sputum specimens

Yeast species	Frequency (%)
Candida albicans	21 (55.2)
Candida dubliniensis	11 (28.9)
Candida glabrata	04 (10.5)
Candida tropicalis	01 (2.6)
Cryptococcus neoformans	01 (2.6)
Total	38 (100)

Conclusion

The second most common isolated fungi were *Aspergillus* species (4%) including *A. flavus* and *A. fumigatus*. Increase of the outbreak of pulmonary invasive aspergillosis has been reported in immunosuppressed patients(18-20). In Iran, for the first time Bajoghli and Ashrafi reported aspergilloma of the upper lobe of the right lung caused by *Aspergillus fumigatus* in 1353(21). Among other isolated fungi at this work, Mucoral fungi were detected. Similar to the findings of this work, pulmonary mucormycosis has been reported mostly in patients with blood malignancy or bone marrow transplant receivers; mortality rate in this form of disease has been reported to be 76% (7, 22).

Totally, in this study, the most common isolated fungi were *Candida* and *Aspergillus* which complied with previous findings (34-36). Since *Candida* is a member of natural body microflora and *Aspergillus* is a common environmental fungi, these findings could be justified. It supposed to be a conditional colonization in the upper respiratory tract for some isolated *Candida* and a probable infection by *Aspergillus*

studied species. As you know, *Pneumocystis jirovecii* is an atypical fungus; acquired by the airborne route. The main risk factor for developing this mycosis is HIV infection, particularly when CD4 levels are below 200cells/mm^3. It can also occur in other cell-mediated immunosuppressive states common to other mycoses.

Therefore, it is important to identify these agents. Among available methods for the identification of common and important yeast species, culturing on chromagar medium is very simple and at the same time reliable. Since the first introduction of this medium, several studies have been performed on the evaluation of their efficiency or their application in epidemiological investigations.

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