RESEARCH ARTICLE

Invasive fungal disease in critically ill and Immunocompromised patients

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Abstract

Objective: To study the frequency and type of invasive fungal disease in critically ill and immunocompromised patients.

Method: The prospective, cross-sectional, descriptive study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January 2017 to December 2020, and comprised pathological samples from immunocompromised and critically ill patients for fungal culture. Data regarding demographics, comorbidities, results of direct microscopy and fungal culture was recorded. Data was analysed using SPSS 22.

Results: Of the 8285 patients' specimens, 4722(57%) belonged to males and 3563(43%) to females. The mean age of the patients was 48.32±5.42 years (range: 14-98 years). Out of total 8285, 3465(41.82%) were related to blood, 2640(32%) endobronchial washing, 837(10%) sputum, 623(7.5%) tissue, 332(4%) body fluids, 288(3.5%) bronchoalveoar lavage and 100(1.2%) cerebrospinal fluid. Aspergillus flavus (20.7%) and candida albicans (14.5%) were the two most commonly isolated fungal species.

Conclusion: A high index of suspicion for invasive fungal disease should be maintained in immunocompromised and critically ill patients.

Keywords: Invasive fungal disease, Critically ill, Immunocompromised, Candida spp., Aspergillus spp.

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Introduction

Fungi are one of the most abundant organisms on earth, scattered in the air, soil, water or on dead matter, and surviving as parasites or in symbiosis with organisms.[1] Spores of fungi are inhaled during every breath, which may result as either no disease at all or manifest itself as diseases ranging from simple allergy to life-threatening invasive fungal disease, depending upon the immune status of the host. Invasive fungal disease is described as the presence of yeast or moulds in a tissue specimen or sample from a sterile site, like blood, cerebrospinal fluid (CSF), ascitic fluid, pleural fluid or respiratory samples, like sputum etc.[2]

Fungi are typically opportunistic in nature, with fungal infections typically affecting immunocompromised patients.[3] It can result in a fatal outcome in a significant proportion of such patients.[4] With the advent of antiretroviral medication, increase in the quantity and quality of intensive care units (ICUs) and development of procedures such as stem cell or organ transplant, the number of immunocompromised patients have increased manifold and such patients have longer lives than before. Thus, the occurrence of invasive fungal infections is also on the rise, owing to the rising numbers of patients suffering from neutropenia, acquired immunodeficiency syndrome (AIDS), haematological malignancies or immunosuppression due to any cause.[5] Traditionally, aspergillus species (spp), candida (C.) albicans, cryptococcus and pneumocystis have been considered the most frequent causative agents, but non-albicans candida spp and a variety of other fungi, like fusarium spp, are becoming more and more common now.[6] The organ most frequently affected by fungal infection is the lung, with the disease depicting in a multitude of symptoms, most likely as fever, cough, haemoptysis or chest pain.[7,8]

As many as 6 cases per 100,000 persons are affected by fungal infections annually, with only half of them being detected, making it an important but neglected reason for death in critically ill cases.[9] In Pakistan, an estimated 3.28 million people suffer from serious fungal infections annually with candidaemia, invasive candidiasis, mucormycosis and invasive aspergillosis making the bulk of the cases.[10] Diagnosis of invasive fungal infection is still dependent primarily upon microbiological and histological techniques, with radiological techniques providing an ancillary role.[11] Resistance to antifungal medication is aggravating this dilemma, which results from irrational repeated use of these drugs.[12]

The current study was planned to assess the frequency and type of invasive fungal disease in critically ill and immunocompromised patients.

Patients and Methods

The prospective, cross-sectional, descriptive study was

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conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from January 2017 to December 2020. After approval from the institutional ethics review board, the samples were collected using non-probability consecutive sampling technique. All invasive fungal Culture specimens received at AFIP during this period were included in this study.

Those included were critically ill and immunocompromised patients irrespective of age and gender. Critically ill patients meant those admitted to the ICU. Immunocompromised patients included individuals with any cause of congenital or acquired immunodeficiency, such as patients on steroids or chemotherapy, AIDS patients, bone marrow or organ transplant recipients, generalised malignancy, chronic renal failure patients or patients with lung disease. Samples of immunocompetent outdoor patients, those with known systemic or localised fungal disease or patients using antifungal medication at the time were excluded to overcome confounding factors and bias.

Samples collected related to tissues, blood, CSF, sputum, bronchoalveoar lavage (BAL), endobronchial washing (EBW) and body fluid specimens which included specimens other then blood and CSF, like pleural, synovial and peritoneal fluids All invasive sterile specimens submitted for fungal cultures were included.

All samples received were dealt with in a biosafety cabinet. Wet mount for fungal hyphae and pseudohyphae were observed to correlate with culture findings and establishing the significance of isolate grown in culture.

All tissue specimens were inoculated on Sabouraud agar, Sabouraud dextrose agar (SDA) with chloramphenicol and SDA with actidione via three-point inoculation technique to rule out contamination. All plates were incubated at 22-26°C.

All fluid specimens, including CSF, were inoculated on Sabouraud agar and SDA with chloramphenicol via three-point inoculation technique to rule out contamination. All plates were incubated at 22-26°C. India ink staining was done in CSF samples to look for budding yeast cells cryptococcus neoformans.

Respiratory tract specimens were inoculated on Sabouraud agar and SDA with chloramphenicol. Plates were incubated at 22-26°C.

All specimens were incubated for 4 weeks. All plates were visualised daily for the first week, and then twice every week for the next 3-4 weeks, until growth was visualised.

All blood culture vials received for fungal culture were incubated at 35-37°C in an automated blood culture system. Vials were removed once they gave positive signal. Subculture was done on Sabouraud agar, SDA with chloramphenicol, and Sheep blood agar. Plates were incubated at 22-26°C.

Once the culture yielded growth of mould, lactophenol cotton blue preparation was performed and morphologies were matched according to the guidelines of the American Society of Microbiologists.[13]

If the culture yielded growth of yeast, then Chrome agar, API Aux (Analytical Profile Index for the Identification of Yeasts) and VITEK (Automated system used for identification and susceptibility testing of microorganism) were applied for specie identification.

All culture plates were kept in transparent zip-lock bags and then incubated to prevent contamination and potential laboratory exposure.

The samples were reported as "no fungal growth seen" on the basis of absence of growth even after 04 weeks of incubation.

All the results of microscopic examination as well as the fungal culture on the samples along with fungal species present were documented. Data was analysed using SPSS 22. Frequencies and percentages were calculated for different species in the positive specimens.

Results

Of the 8285 patients' specimens, 4722(57%) belonged to males and 3563 (43%) to females. The mean age of the patients was 48.32±5.42 years (range: 14-98 years). Most of the samples belonged to patients aged 40-59 years (43.1%). Most common comorbidities among 8285 patients included diabetes mellitus 2601 (31.4%), chronic kidney disease (CKD) 1888 (22.8%), chronic lung disease 969 (11.7%) and malignancy 778(9.4%).

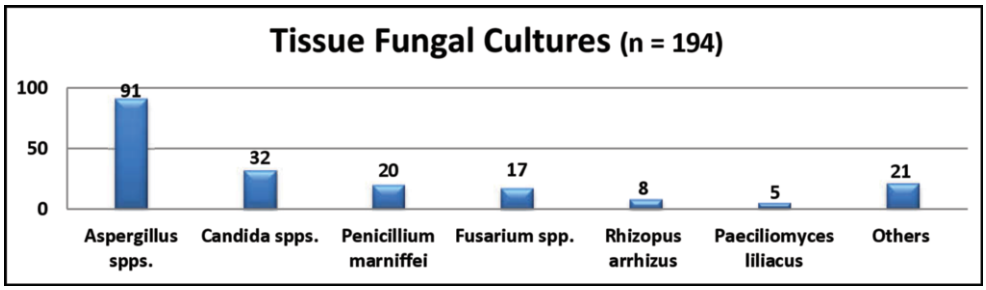

Figure-1: Distribution of fungal species (sppspp) in tissue cultures.

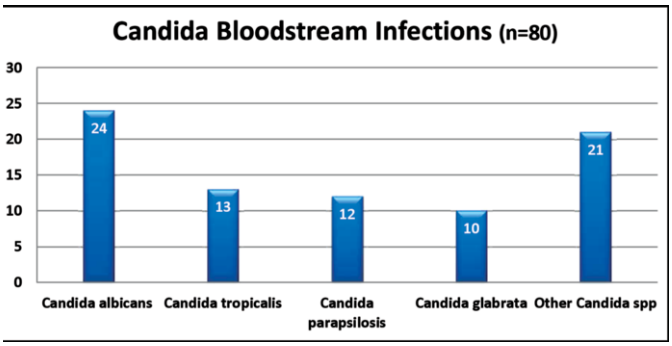

Figure-2: Distribution of candida specie (sppspp) in blood cultures.

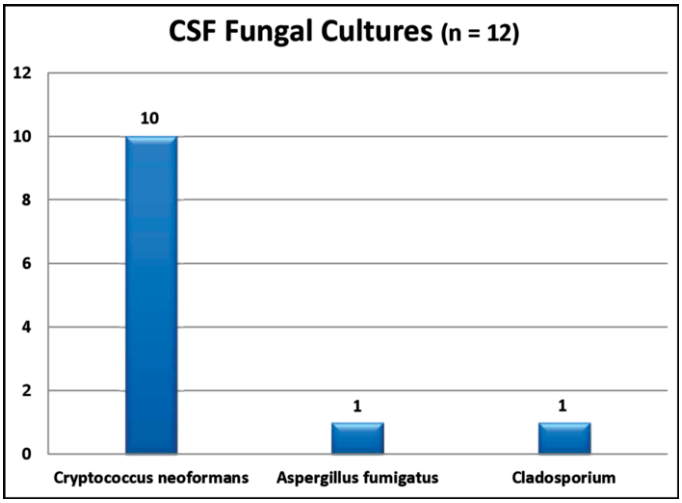

Figure-3: Distribution of fungal species (spp) in cerebrospinal fluid (CSF) cultures.

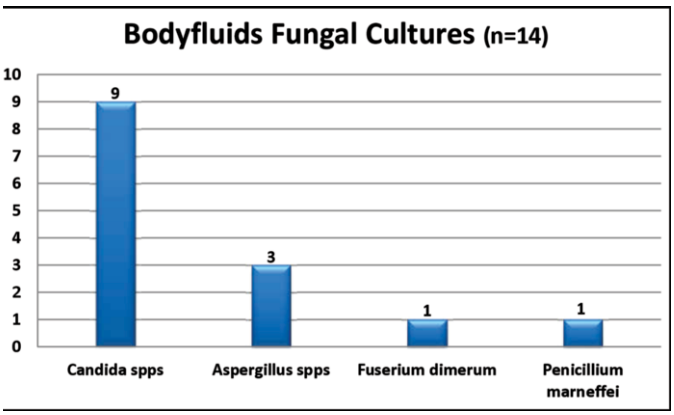

Figure-4: Distribution of fungal species (spp) in bodyfluid cultures.

Out of 8285 specimens, 3465 (41.82%) related to blood, 2640(32%), EBW 837(10%) sputum, 623(7.5%) tissue, 332(4%) body fluids, 288(3.5%) BAL and 100(1.2%) CSF. Aspergillus spp., were the most common 91/194(46.9%), followed by Candida spp., 32/194(16.5%) and Penicillium marneffei 20/194 (10.3%) (shown in Figure-1). Among the Aspergillus species, A. flavus were 50/91 (54.9%), followed by A. fumigatus, 32/91(38.9%). Amongst the Candida spp,

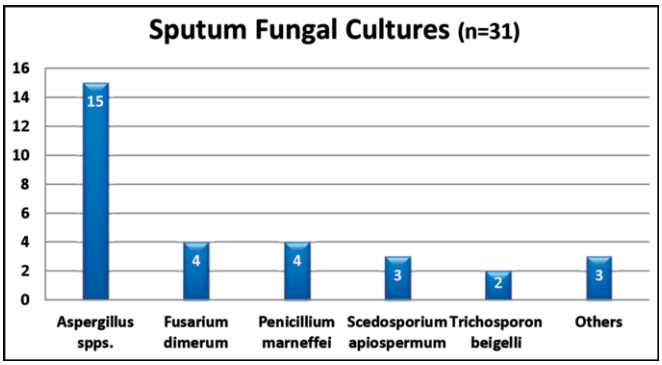

Figure-5: Distribution of fungal species (spp) in sputum cultures.

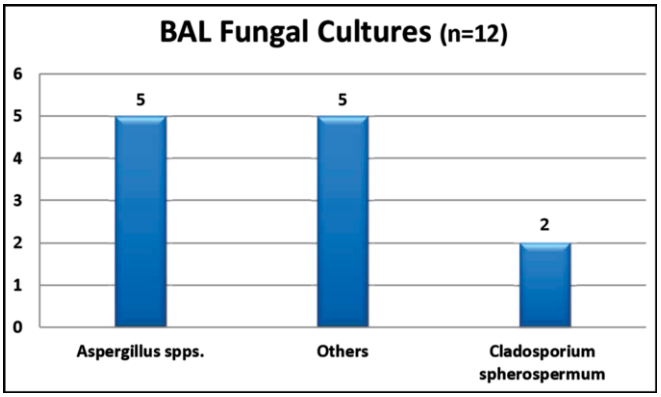

Figure-6: Distribution of fungal species (spp) in bronchoalveoar lavage (BAL) cultures.

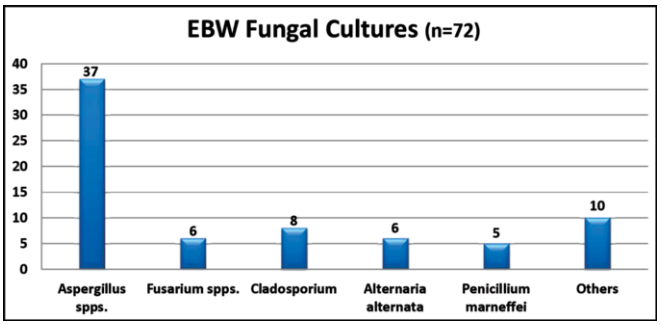

Figure-6: Distribution of fungal species (spp) in bronchoalveoar lavage (BAL) cultures.

C. albicans made majority of the cases 30/32(93.8%).

Of the 3465(41.82%) blood specimens, 80 (2.6 %) were positive. The most common was C. albicans, 24(30%), followed by C. tropicalis 13(16.2%) (Figure-2).

Distribution of CSF samples (shown in Figure-3), body fluid (shown in Figure-4), sputum (shown in Figure-5), BAL (shown in Figure-6) and EBW (shown in Figure-7) were also studied.

Discussion

Fungi are eukaryotic organisms that possess a cell wall made up of chitin. They have emerged as a significant

cause of infection affecting immunocompromised patients, leading to considerable mortality, with the burden reaching more than 1.6 million, as much as that of tuberculosis (TB) and more than three times that of malaria.[14] This dilemma is more extensive in Asian countries, where they thrive owing to the climate, scarcity of healthcare facilities as well as abuse of steroids and antibiotic.[9] Factors predisposing to invasive fungal disease include congenital deficiency of the immune system, granulocytopenia <0.5x109/L lasting for >10 days, allogeneic stem-cell transplantation, intake of immunosuppressive medication and corticosteroids, patients in ICU and patients having structural pulmonary disease and/or complicated influenza.[15] Early and reliable diagnosis assumes cardinal importance, as a vital association exists between prompt reliable diagnosis and early management of invasive fungal disease and better outcome of the patients at risk.[16]

In the current study, 8285 samples were included, and 5% of them were positive. A. flavus (20.7%) and C. albicans (14.5%) were the two most commonly isolated species. A. flavus was most commonly encountered amongst the tissue samples, BAL specimens and EBWs. C. albicans was most frequent in the blood samples and body fluids, while cryptococcus neoformans was most repeatedly found in CSF samples.

A similar prospective study at a tertiary healthcare institution in India on invasive fungal infections in critically ill patients showed 15% cases suffering from invasive fungal disease, with invasive aspergillosis being the most common, followed by invasive candidiasis.[17] Similarly, a study investigating fungal infections in adult patients on extracorporeal life support included data from more than 300 international centres to show that 10.8% harboured fungal infection with aspergillus involvement the being most common, followed by candida invasive bloodstream infection.[18] Another study was organised in Iran concerning 400 BAL samples for invasive fungal disease, which pointed out that A. flavus was the most common isolate, followed by A. fumigatus and A. niger.[19]

Exhaustive efforts to locate a local research on this topic did not prove fruitful. The findings are mostly in line with some similar international studies quoted above. Minor differences in results are likely due to dissimilarity in the sample size or diversity in inclusion criteria. As there is scarcity of published local research on the topic, the current study will set the benchmark in this direction. However, the study does have its limitation as the findings relate to a single centre. Also, advanced techniques, like polymerase chain reaction (PCR) or serological tests, like galactomannan and beta-D-glucan, were not used which may have resulted in a number of false negative cases. However, the two tests are newer pathways which need to be further evaluated and were beyond the scope of the current study.

Limitation of study includes that sample size was not calculated.

Conclusion

Invasive fungal infections continue to be cause significant morbidity and mortality in immunocompromised patients around the world. A high index of suspicion for invasive fungal disease should be maintained in such patients. Prompt diagnosis and effective appropriate medication is essential to improve chances of survival.

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Conflict of Interest: None.

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