Original Article

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Invasive fungal infections in critically ill patients: A prospective study from a tertiary care hospital in India

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Summary **Invasive fungal infections (IFI) are commonly seen in immunosuppressed individuals but their epidemiology in critically ill patients has not been well described. The aim of this study was to determine the frequency, risk factors and outcome of invasive fungal infections in a medical intensive care unit. A prospective observational study was carried out between August 2016 and March 2018 in the medical intensive care unit. Patients above the age of 14 years with endotracheal intubation and/or central venous catheter for at-least three days and sepsis (not responding to 48 hours of intravenous antibiotic therapy) were included in the study. Suitable samples were collected and were subjected to fungal diagnostics. Invasive fungal disease was defined according to standard guidelines. Of the 100 recruited patients, a total of 11 patients had invasive aspergillosis, three patients had invasive candidiasis and one patient had both invasive aspergillosis and mucormycosis. IFI was more commonly seen in patients with auto-immune diseases (p = 0.002, odds ratio-10.13 (95% CI: 2.3-44)). A mortality of 73% was observed in patients with IFI. In conclusion, IFI, especially aspergillosis is grossly under-reported in critical settings. Early suspicion, thorough investigation and timely diagnosis may alleviate patients of significant mortality and morbidity.**

Keywords: *Aspergillus*, *Candida*, mucormycosis, intensive care unit

1. Introduction

The course of hospital stay in critically ill patient is often complicated by hospital acquired infections. While bacterial infections are known to be very common in critical care settings, the incidence of invasive fungal infections (IFI) is also on the rise. Invasive candidiasis has been found to be the commonest IFI in such settings (*1-3*). Majority of these cases are ascribed to the rampant use of broad spectrum antibiotics and disruption of normal skin and mucosal barriers with multiple devices (*4-6*). Invasive aspergillosis is the second most common IFI. Although, it is predominantly reported in hematopoietic stem cell transplant recipients (*7*) and solid organ transplant recipients (*8*), invasive aspergillosis is increasingly being reported in intensive care units (ICU). ICU patients are at high risk for colonization with *Aspergillus* because of defective mucosal clearance and mechanical ventilation. Hospital environment and construction works have been implicated as the source of spores (*9,10*). Other fungal infections like those caused by *Mucorales* and *Scedosporium* are relatively rare in the medical ICUs and seen mostly in patients with background immunosuppression (*11,12*). While there is enough literature regarding the incidence and outcome of fungal infections in immunosuppressed populations (transplant recipients/malignancy/human immunodeficiency infection virus), the literature on the epidemiology of fungal infections in critically ill patients is scarce. The primary objective of the study was to therefore, determine the frequency, risk factors and outcome of invasive fungal infections in a medical intensive care unit.

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2. Methods

A prospective observational study was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments after taking approval from the Institute's ethical committee. The study was carried out in the medical intensive care unit at a tertiary care hospital in New Delhi, India between August 2016 and March 2018. All patients above the age of 14 years with endotracheal intubation and /or central venous catheter for 3 or more days and sepsis (not responding to 48 hours of intravenous antibiotic therapy) were included in the study after taking informed consent. Those patients already diagnosed with an IFI before enrolment, those who were neutropenic at the time of enrolment, those who were already diagnosed with human immunodeficiency virus infection or malignancy, transplant recipients and those who had received antifungals in the two weeks prior to enrolment were excluded from the study.

The recruited patients were evaluated for risk factors implicated in the development of IFI. Suitable samples for diagnostic purposes like bronchio-alveolar lavage (BAL)/mini-BAL, blood, sputum, urine, cerebrospinal fluid (CSF), ascitic fluid and pleural fluid were collected and subjected to potassium hydroxide (KOH) mount, gram stain, India ink preparation and fungal cultures (BACTEC, Becton Dickinson, USA). Each culture set was examined daily for first week & twice weekly thereafter for next three weeks. Cultures were deemed negative if there was no growth after four weeks of incubation. Positive cultures were identified and speciated based on the culture morphology and microscopic features. Antifungal susceptibility was done for yeast isolates using micro-broth dilution method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Additionally, those with features suggestive of invasive aspergillosis were subjected to serum and BAL galactomannan (Platelia *Aspergillus* enzyme immunoassay, Bio-Rad Laboratories, Munich, Germany) tests as per manufacturer instructions. A complete urine analysis, chest X-ray and bacterial cultures were done for all the patients. A computed tomography (CT) of the chest was also done, wherever indicated.

IFI was defined and categorized into proven, probable and possible based on the revised European Organization for the Research and Treatment of Cancer/ Mycoses Study Group (EORTC/MSG) guidelines (*13*). In cases of suspected invasive pulmonary aspergillosis, where the patients could not be classified according to EORTC/MSG guidelines, algorithm given by Blot *et al*. was used to further classify them into putative aspergillosis and colonization (*14*). Putative aspergillosis was also included in the IFI category.

Statistical analyses were performed using the Stata version 12.1. Categorical variables were expressed as percentage and continuous variables were expressed as mean ± standard deviation. Patients with IFI and without IFI were compared using appropriate statistical tests. Odds ratios (ORs) with 95% confidence interval (CI) were calculated. A *p*-value of less than 0.05 was considered significant.

3. Results

A total of 100 patients who satisfied the inclusion and exclusion criteria were recruited in the study. Of these, 53 patients were male and 47 were female. Most patients were between the age range of 60 and 79 years (41%). The mean age for the study population was 48.9 ± 19.8 years. Pneumonia (39%), acute febrile illness (14%), urinary tract infection (13%), meningoencephalitis (6%) and gastroenteritis (5%) were the most common primary diagnosis in these patients. Diabetes (29%), chronic kidney disease (18%), chronic lung disease (15%), auto-immune disease (9%) and chronic liver disease (6%) were the most common co-morbidities in the recruited patients.

A total of 15 patients were diagnosed with IFI amounting to a frequency of 15% (95% CI 7.88-22.12). Of the 15 patients with IFI, 11 patients had invasive aspergillosis, three had invasive candidiasis and one patient had both invasive aspergillosis and mucormycosis. A total of 123 blood cultures were sent, of which three cultures grew *Candida* spp. (*Candida albicans*, *Candida krusei* and *Candida tropicalis*). *Candida krusei* was resistant to fluconazole. Candida albicans and Candida tropicalis were sensitive to all the tested drugs (amphotericin B, voriconazole, fluconazole, caspofungin and micafungin). Out of the 178 mini-BAL cultures sent, 23 samples tested positive for molds. A total of 12 patients (Probable-6, Putative-6) satisfied the criteria for invasive fungal infection. Of these twelve patients, ten patients were culture positive: *A. fumigatus* (n = 5), *A. flavus* (n = 1), *A. terreus* (n = 1), *A. fumigatus* + *A. flavus* (n = 1), *A. fumigatus* + *A. terreus* (n = 1), *A. flavus* + *Lichtheimia corymbifera* (n = 1). Two patients satisfied the criteria for IFI but were culture negative. Of the 140 urine cultures sent, 18 cultures were positive for *Candida* spp. In absence of suggestive clinical findings and concurrent positive blood cultures, these were regarded as colonization. Mean time taken for culture positivity from the day of admission for blood, mini-BAL and urine was 15.6, 8 and 8 days respectively.

There was no statistically significant association between occurrence of IFI and baseline sequential organ failure assessment (SOFA) score, acute physiology and chronic health evaluation (APACHE) II score, duration of mechanical ventilation, duration of central venous catheterization, duration of ICU stays and duration of antibiotics (Table 1). Risk factors other than presence of auto-immune conditions (p = 0.002, odds ratio-10.13

Table 1. Comparison of risk factor between patients with/without Invasive fungal infections (IFI)

Variable	In patients without IFI (Median, IQR), *N* = 85	In patients with IFI (Median, IQR), *N* = 15	*P* value
SOFA score	11 (9 – 13)	10 (5 – 14)	0.55
APACHE II score	22 (17 – 27)	25 (13 – 28)	0.97
Mechanical ventilation (days)	11 (7 – 22)	10 (8 – 18)	0.88
Central venous catheterization (days)	11 (8 – 20)	13 (10 – 22)	0.37
Length of stay in Intensive care unit (days)	12 (7 – 20)	13 (8 – 19)	0.57
Duration of broad spectrum antibiotics (days)	19 (11 – 31)	20 (11 – 25)	0.97

Table 2. Occurrence of Invasive fungal infections (IFI) with respect to various risk factors

Variable	Risk factor absent (%)	Risk factor present (%)	*P* value
Chronic kidney disease	15.85%	11.11%	1.00
Chronic lung Disease	12.94%	26.67%	0.23
Chronic liver disease	13.83%	33.33%	0.22
Autoimmune conditions	10.99%	55.56%	0.003
Diabetes mellitus	15.49%	13.79%	1.00
Hemodialysis	16.92%	11.43%	0.57

(95% CI: 2.3-44)) did not affect the rate of IFI (Table 2). Out of 15 patients with IFI, 11 patients (73.3%) expired whereas 55 (64.7%) expired out of the 85 patients without IFI.

4. Discussion

Invasive fungal infections in non-immunosuppressed critically ill patients is a less explored area with most studies concentrating on invasive candidiasis (candidemia and deep seated tissue infections) alone. Incidence of candidemia in literature ranges from 6.51 to 54 per 1,000 patients in various studies, most of which are from European countries (*16-18*). The mean interval from ICU admission to the occurrence of candidemia in our patients was 15.67 days. Although, studies from Europe report similar findings, this duration was reported as 8 days from an Indian study (*18-20*).

According to a systematic review, 80% of IFI in critically ill patient are due to *Candida* spp. while only 0.3-19% of these infections are caused by invasive aspergillosis (*21*). Two studies in Italy reported an incidence of invasive aspergillosis of 2.3 per 1,000 admissions and 6.8 per 1,000 admissions respectively (*16,17*). Meersseman *et al*. conducted a multi-center retrospective study in Belgium and reported a frequency of invasive aspergillosis in critically ill patients without malignancy to be 6.9% (*22*). The frequency of aspergillosis in most studies may be grossly under-reported because of the uncertainty in diagnostic criteria. In our study, out of 15 cases of IFI, only three were caused by *Candida* spp. and the rest by *Aspergillus* spp. This differential distribution might have been due to *i*) Use of expanded criteria for diagnosis of invasive aspergillosis- EORTC/MSG criteria has long been used for diagnosis of invasive aspergillosis but it is often difficult to classify invasive aspergillosis in non-neutropenic critically ill patient with these criteria alone. To resolve this issue, we used the Blot's criteria in those patients where classification could not be done based on EORTC/MSG criteria (Probable-6, Putative-6, colonization-9), *ii*) lack of traditional risk factors for invasive candidiasis in medical ICUs like total parenteral nutrition and gastro-intestinal surgery, *iii*) Presence of risk factors for aspergillosis like chronic lung diseases, chronic liver disease and auto-immune disorders, *iv*) ubiquitous presence of *Aspergillus* spores coupled with constructional activities in hospital (*9*).

The mean duration of ICU stay before positivity of mini-BAL culture (calculated from the day of admission in ICU) in patients with invasive aspergillosis in our study population was eight days. Various other studies have reported this duration to be in the range of 4 to 15.6 days (*17,23,24*). Most common species isolated from the mini-BAL of patients with invasive aspergillosis was *Aspergillus fumigatus* (70%) followed by *Aspergillus flavus* (30%) and *Aspergillus terreus* (20%). Most studies from Europe revealed *Aspergillus fumigatus* (82-92%) as the commonest fungus causing invasive Aspergillosis (*17,24*).

Statistically significant relationship was established between autoimmune diseases and occurrence of IFI. Out of the nine patients admitted with auto-immune diseases, five patients developed invasive aspergillosis ((p = 0.003) with an odds ratio of 10.125 (p = 0.002)). Patients with chronic lung disease (p = 0.23) and chronic liver disease (0.22) also had increased frequency of IFI but this finding was not statistically significant. Meersseman et al. found the use of corticosteroids for more than 3 weeks and chronic liver disease as an important risk factor for invasive aspergillosis in critically ill patients (*22*). Garnacho-Montero et al. also concluded that COPD and use of corticosteroids was associated with positive culture for

Aspergillus from lower respiratory samples (*25*).

A crude mortality rate of 30-81% and an attributable mortality of 5-71% has been reported for invasive candidiasis in various studies whereas a mortality rate of up to 80% has been reported for invasive aspergillosis (*2,3,21-23,25*). Owing to the fact that ours is an apex care center receiving referral from most parts of India, the study population had a higher mean age, high frequency of co-morbidities and high base-line APACHE-2/SOFA scores. This was probably the reason for high mortality across both the groups.

The incidence of IFI, particularly invasive aspergillosis, is grossly under-reported in critical care settings. Early suspicion and thorough investigation, especially in presence of established risk factors such as use of immunosuppressive agents and chronic lung diseases, should be carried out. These infections are associated with high mortality. Institutional measures like infection control and anti-microbial stewardship are the need of the hour.

Limitations

Beta-d glucan test could not be done in these patients which has a higher sensitivity than blood culture for making a diagnosis of invasive candidiasis. This may have led to under-reporting of invasive candidiasis cases.

Declarations

Ethics approval was taken from the institute's ethics committee and informed consent to participate in the study was taken from all patients/ surrogates. Competing interests: The authors declare that they have no competing interests. Funding: The authors have no funding source to declare. Acknowledgements: The authors thank the medical and nursing staff of the medical intensive care unit.

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