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Invasive Fungal Infections in Critically Ill Patients: A Prospective Study from a Tertiary Care Hospital in Bangladesh

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Abstract

Introduction: *Invasive fungal infections (IFI) are common nosocomial infections in immunosuppressed individuals.* ***Method:*** *It was a cross-sectional and observational study. A total of 77 peripheral venous blood samples were collected from clinically suspected IFI patients from the intensive care unit (ICU) of Bangabandhu Sheikh Mujib Medical University (BSMMU). The study was conducted from December 2022 to August 2023.* ***Result:*** *A total of 77 critically ill patients with a high risk of invasive fungal infection (IFI) were classified based on blood culture into three groups: no fungemia (41 patients with proven bacterial sepsis), suspected fungemia (25 patients with negative blood culture), and definite fungemia group (11 patients with culture-proven fungemia). 10/54 presented Candida spp. and 1/54 presented Aspergillus spp. Hospitalization for more than 3 weeks, the use of a central device, persistent thrombocytopenia, and CRP levels were significantly higher in the definite fungemia group compared to the other two groups. In contrast, mechanical ventilation lasting more than 1 week was significantly higher in the definite fungemia group compared with the no fungemia group. IFI was more commonly seen in patients with autoimmune diseases (p = 0.002, odds ratio 10.13, 95% CI: 2.3-44). The majority of cases were from the age group of ≥60 years, predominantly male.* ***Conclusion:*** *Invasive fungal infection is grossly underreported in critical settings. Early suspicion, thorough investigation, and timely diagnosis may alleviate patients of significant mortality and morbidity.*

Keywords: *Invasive fungal infections, risk factors, fungemia.*

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Introduction:

Every year, fungi cause at least 13 million illnesses and 1.5 million fatalities worldwide, mostly in those with weakened immune systems[1]. An estimated 1.9 million patients get acute invasive fungal infections (IFI) annually, while 3 million persons globally are thought to have severe chronic fungal infections[1]. IFI has become more common in recent years, primarily among hospitalized patients. The majority of the opportunistic infections that cause IFIs are Aspergillus species, Candida, and Cryptococcus[2]. Although the prevalence of candidemia varies greatly from nation to nation, a study indicated that 8100 instances of the disease occur annually in Bangladesh at a conservative incidence of 5 per 100,000. In Bangladesh, invasive aspergillosis (IA) is seldom acknowledged among other severe fungal illnesses[3]. In Bangladesh, there are an estimated 2,481,444 people with COPD (7.2%) out of 34.5 million persons over 40[4]. Assuming that 1.3% of these patients acquire IA and 13% of them are hospitalized annually[4]. According to the previously mentioned facts, aspergillosis and invasive

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candidemia represent a significant burden in Bangladesh. The most prevalent IFI in intensive care units is invasive candidiasis[5,6,7]. Most of these cases are attributed to the widespread use of broad-spectrum antibiotics and the various devices that disturb normal skin and mucosal barriers[8,9,10]. In terms of IFIs, invasive aspergillosis is the second most prevalent. Invasive aspergillosis is increasingly being documented in intensive care units (ICUs), despite the fact that it is primarily observed in patients of solid organ transplants[11] and hematopoietic stem cell transplants[12]. ICU patients are particularly vulnerable to Aspergillus infection due to compromised mucosal clearance and mechanical breathing. Spores have been linked to construction sites and hospital environments[13,14]. Other fungal infections, such as those brought on by Scedosporium and Mucorales, are comparatively uncommon in medical intensive care units and are typically observed in patients who have a history of immunosuppression[15,16]. The incidence and risk factors of fungal infections in immunosuppressed groups (transplant recipients, cancer patients, and HIV) are well documented, but there is a dearth of information on the epidemiology of fungal infections in critically sick patients. Therefore, identifying the prevalence and risk factors of invasive fungal infections in a medical intensive care unit was the study's main goal.

Materials and Methods:

This is an observational and cross-sectional study conducted in a tertiary care hospital in a time span of[9] months, from December 2022 to August 2023. Peripheral venous blood was collected from the patients admitted to the intensive care unit of Bangabandhu Sheikh Mujib Medical University (BSMMU). Laboratory work was performed in the Department of Microbiology & Immunology, (BSMMU). Clinically suspected patients of IFIs were admitted to the intensive care unit of BSMMU. **Inclusion criteria were** Clinically suspected patients of IFIs with any of the following factors were included in this study. The factors are Neutropenia (<0.5x 10^9 neutrophils/L), Use of corticosteroid for a prolonged time (minimum dose of 0.3 mg/kg/day of prednisone equivalent, for >3 weeks) and Fever refractory to at least 3 days of appropriate antibiotics/fever relapsing after a period of defervescence of at least 48 hours while still receiving antibiotics. **Exclusion criteria were** Participants were not be enrolled if they- Had any bacterial/parasitic infections, Were on the regimen for anti-fungal therapy.Relevant data were collected from patients or their attendants or the clinical history records and investigations of the patients in a predesigned data collection sheet. Results obtained from laboratory methods were recorded in a separate data collection sheet. **Procedures in the laboratory:** Peripheral venous blood was collected under aseptic conditions using a sterile disposable syringe tagged with a butterfly needle after preparing the patient's skin with, at first 70% alcohol and hereafter with 1% tincture iodine[17]. A total of 10 ml of blood was collected and inoculated into an automated blood culture bottle. **Blood culture and identification of yielding fungi:** Inoculation of 10 ml of blood into the BD-BACTECTM Plus Aerobic/F blood culture bottle after disinfection of the head of the bottle with 70% alcohol[18]. Then the blood culture bottle was inserted into the BD-BACTECTM FX40 (Becton, Dickinson and Company, Sparks, MD 21152, USA) blood culture machine for incubation at 37^0 C temperature for 1-5 days according to the manufacturer's instruction[19]. After showing positive indicators on the machine; with all necessary aseptic precautions, isolation of microorganisms was done by sub-culturing a small amount of liquid media on Sabouraud dextrose agar (SDA) media, blood agar media, and MacConkey agar media and incubated at 37^0C for 24 hours aerobically, up to 4-5 days for SDA media, as the maximum time required for Candida and Aspergillus species to grow in SDA media is 4-5 days[20]. A smear was also made from growth on subculture media and Gram stain preparations were performed. All the bottles and the culture media were discarded after 7 days according to the proper safety procedure[21]. Identification of organisms was performed using colony morphology, wet film, Gram staining, microscopy, and biochemical tests. **Ethical approval:** This study was ethically approved by the Institutional Review Board (IRB), BSMMU (NO. BSMMU/2022/12411) on 12/12/2022. **Statistical analysis:** Data analysis was done using the SPSS software package version 27 (Strata Corporation, College Station, Texas). The Kolmogorov-Smirnov test was used to check for normal data distribution. Categorical data were described using numbers and percentages. Continuous data were described using median, mean, range, and standard deviation. The chi-square test was used for categorical variables, one-way ANOVA for Gaussian data, and Kruskal-Walls test for non-Gaussian data to compare between three or more groups.

Results:

A total of 77 critically ill patients with suspected IFI were enrolled during the study period; the study flow chart (Figure 1) shows how the patients were categorized into groups. A total of 77 blood samples from patients with invasive fungal infections who were clinically suspected were used in this investigation. As illustrated in a pie chart in Figure 2, of the blood samples from 77 individuals with clinically suspected invasive fungal infections, 11 (14%) tested positive for fungus, 41 (53%) tested positive for bacteria, and 25 (33%) tested negative for growth. Gram-negative bacteria were more common than Gram-positive bacteria in the blood samples of 77 patients with clinically suspected invasive fungal infections (32/54 and 11/54, respectively). The organisms that were grown were Proteus spp. (2/54), Pseudomonas aeroginosa (3/54), coagulase-negative Staphylococcus [9/54], Staphylococcus aureus [3/54], Klebsiella spp. (14/54), and Escherichia coli (12/54).

Additionally, 1/54 had Aspergillus spp. and 10/54 presented Candida spp. Both blood cultures showed mixed bacterial and fungal growth. Hospitalization for more than 3 weeks, the use of a central device, persistent thrombocytopenia, and CRP levels were significantly higher in the definite fungemia group compared to the other two groups. In contrast, mechanical ventilation >1 week was significantly higher in the definite fungemia group when compared with the no fungemia group only (Table I). The most frequent main diagnosis among these patients was pneumonia (39%), followed by acute febrile illness (14%), urinary tract infection (13%), meningoencephalitis (6%), and gastroenteritis (5%). The most prevalent co-morbidities among the patients that were recruited were diabetes (29%), chronic kidney disease (18%), chronic lung disease (15%), auto-immune illness (9%), and chronic liver disease (6%). The rate of IFI was unaffected by risk factors other than the existence of auto-immune disorders (p = 0.002, odds ratio-10.13 (95% CI: 2.3-44)) (Table II). Table III shows the age group distribution according to gender in the study population. Out of 77 patients, 6 (13.0%) were male and 1 (3.2%) were in the age group of 18-30 years, 6 (13.0%) were male and 3 (9.7%) were female in the age group of 31-40 years, 6 (13.0%) were male and 6 (19.3%) were female in the age group of 41-50 years, 12 (26.0%) were male and 7 (22.6%) were female in the age group of 51-60 years and 16 (35.0%) were male and 14 (45.2%) were female in the age group of ≥60 years. The majority of cases were from the age group of ≥60 years, predominantly male. The mean age for males was 51.97±16.74 years and for females was 57.67±14.79 years.

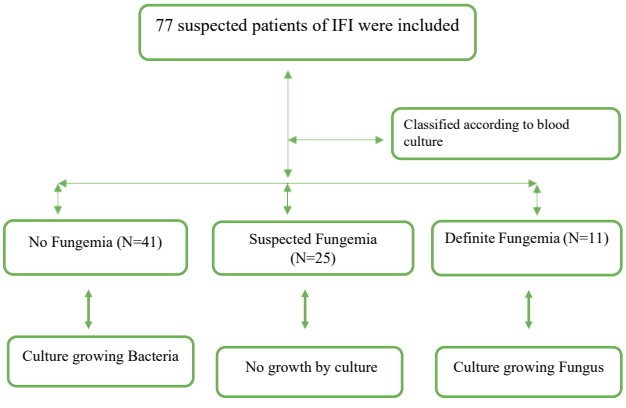

Figure 1: Study flowchart

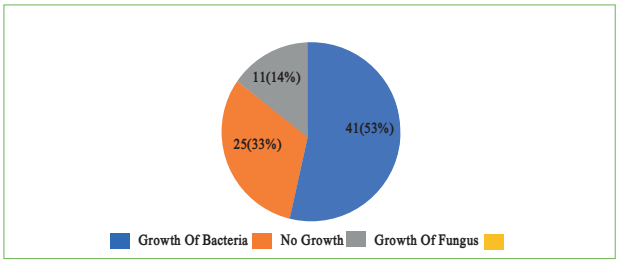

Figure 2: Results of automated blood culture among the study population (n=77)

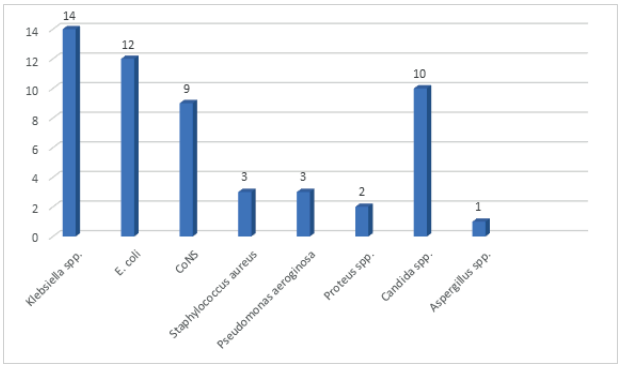

Figure 3: Organisms isolated in automated blood culture

Table I: Clinical and laboratory characteristics of the studied groups

	No fungemia n=41	Suspected fungemia n=25	Definite fungemia n=11	p[a]	p[b]	p[c]	p[d]
Hospitalization >3 weeks	30 (73.2%)	15 (60%)	11 (100%)	0.038	0.265	0.043	0.016
Mechanical ventilation	36 (87.8%)	22 (88%)	11 (100%)	0.049	1	0.041	0.538
Systemic antibiotic exposure >72 h	33 (80.5%)	20 (80%)	9 (81.8%)	1	1	1	1
Surgical intervention	4 (9.8%)	5 (20%)	0 (0.0%)	0.367	0.282	0.567	0.295
Central arterial or venous catheterization	23 (56.1%)	15 (60%)	10 (90.9%)	0.003	0.756	0.040	0.016
Total parenteral nutrition	34 (82.9%)	20 (80%)	11 (100%)	0.355	0.754	0.322	0.295
Persistent thrombocytopenia ($<100\times10^9$/L)	31 (75.6%)	14 (56%)	10 (90.9%)	0.041	0.047	0.420	0.049
*CRP (mg/L)	48 (12-208)	48 (12-112)	96 (12-210)	0.022	0.252	0.024	0.042

*CRP- C- reactive protein

[a] Comparison between all groups

[b] Comparison between no fungemia and suspected fungemia

[c] Comparison between no fungemia and definite fungemia

[d] Comparison between suspected fungemia and definite fungemia

Table II: Occurrence of IFI concerning 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 lung disease	13.83	33.33	0.22
Autoimmune disease	10.99	55.56	0.003
Diabetes mellitus	15.49	13.79	1.00
Hemodialysis	16.92	11.43	0.57

Table III: Age group according to gender distribution in the study population (n=77)

Age group	Male (n=46)	Female (n=31)
18-30 years	6 (13.0%)	1 (3.2%)
31-40 years	6 (13.0%)	3 (9.7%)
41-50 years	6 (13.0%)	6 (19.3%)
51-60 years	12 (26.0%)	7 (22.6%)
≥61 years	16 (35.0%)	14 (45.2%)
Mean age (Years)	**51.97±16.74**	**57.67±14.79**

Discussion:

In critically sick patients, invasive fungal infections continue to cause death and life-threatening consequences despite the

development of novel antifungal medications. Since most research focuses on invasive candidiasis (candidemia and deep-seated tissue infections) alone, invasive fungal infections in critically ill non-immunosuppressed individuals are a less studied topic. Numerous investigations, the majority of which are from European nations, have reported incidences of candidemia ranging from 6.51 to 54 per 1,000 patients in the literature[22,23,24]. A comprehensive analysis found that invasive aspergillosis is responsible for just 0.3–19% of IFI in critically sick patients, but Candida spp. account for 80% of these infections[25]. An incidence of invasive aspergillosis was reported in two Italian studies to be 2.3 per 1,000 admissions and 6.8 per 1,000 admissions, respectively[23,24]. According to a multi-center retrospective study by Meersseman et al. in Belgium, 6.9% of critically sick patients who did not have cancer had invasive aspergillosis[26]. Due to the uncertainty in diagnostic criteria, the frequency of aspergillosis may be significantly underreported in the majority of studies. In this study, out of 11 cases of IFI, only one was caused by Aspergillus spp. and the rest by Candida spp. Candida spp. was isolated in 18.5% of the culture-positive cases. A similar rate of 13.5% was reported by Yang, Cheng, and Lo, in 2006 in Taiwan[27]. As only one case of Aspergillus species (1.8%) was identified it is difficult to compare with other studies, where also isolation rate of mold was relatively low. Distribution of risk factors among the studied patients with clinical suspicion of IFIs revealed a high association of IFIs with central devices, persistent thrombocytopenia, total parenteral nutrition, and higher levels of CRP. This result is consistent with a study where the highest percentage of the studied population had TPN, CV line, NG feeding, and prolonged broad-spectrum antibiotic[27]. There is a statistically significant correlation between the incidence of IFI and autoimmune disorders. Five patients ($p = 0.003$) out of the nine autoimmune disease patients admitted also had invasive candidiasis, with an odds ratio of 10.125 ($p = 0.002$). IFI was also more common in patients with chronic liver illness ($p = 0.22$) and chronic lung disease ($p = 0.23$), although this difference was not statistically significant. Chronic liver dysfunction was identified by Meersseman et al. as a significant risk factor for invasive aspergillosis in critically ill individuals[26]. In the present study, the highest age group was in the age of > 61 years old. This is consistent with the work of Meersseman et al (2004)[26] where most of the cases of invasive fungal infection tend to occur at high age (older than 60 years), and this can be attributed to the increased incidence of invasive mycoses with bipolarity of age in association with diminished immunity and body resistance.

Conclusion:

In critical care settings, the incidence of IFI is glaringly underreported. Particularly in the presence of known risk factors including the use of immunosuppressive medications and autoimmune disorders, early suspicion and careful investigation should be conducted. Antimicrobial stewardship and infection control are examples of institutional actions that are urgently needed.

Conflict of Interest: None.

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