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Research Article

Candidemia in Hematological Patients: A Hospital-Based Study Among Adults in Northern India

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ABSTRACT

Background: Candidemia has emerged as a prevalent fungal bloodstream infection globally, particularly impacting individuals with hematological malignancies who face heightened morbidity and mortality. The objective of this study is to achieve early diagnosis and precise identification of candida species in hematological patients.

Methods: We conducted a study spanning from November 2018 to April 2022, enrolling patients with hematological malignancies. Diagnostic approaches included conventional methods, antigen detection, and PCR for early detection of Candidemia. A comparison of conventional, automated, antigen detection, and PCR methods was performed for diagnosis, followed by antifungal susceptibility testing for treatment guidance in hematological patients.

Results: A total of 229 patients with febrile neutropenia were enrolled, comprising 152 males (66.37%) and 77 females (33.62%), with ages ranging from 10 to 77 years (Mean=33.65, Standard deviation=16.40). The prevalence of Candidemia was 2.1% in this study. Blood culture identified Candidemia in five patients (2.6%), while PCR detected it in nine patients (4.8%). Among the four patients with acute myeloid leukemia (AML), Candida tropicalis (3; 1.60%) and Candida auris (1; 0.5%) were prevalent, alongside one case of pancytopenia with Candida tropicalis (1; 0.5%).

Conclusion: This observational study underscores the significant association between Candidemia and hematological malignancies. Automated diagnostic methods demonstrate higher sensitivity and specificity for species identification. The integration of non-culture methods such as PCR and mannan antigen detection into routine laboratory practices holds promise for early diagnosis and improved treatment outcomes in Candidemia infections.

Keywords: Candidemia; Diagnosis; Hematological Patients; Polymerase Chain Reaction; Treatment.

ABBREVIATIONS

PCR: Polymerase Chain Reaction; Mn: Mannan antigen; A-Mn: Anti-Mannan antibodies; ECIL: European Conference on Infections in Leukemia; DMSO: Dimethyl Sulfoxide; MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization; CLSI: Clinical Laboratory Standards Institute.

INTRODUCTION

Candida, a well-known potentially pathogenic yeast, is a frequent cause of nosocomial bloodstream infections worldwide, often leading to severe fungal infections associated with hospital-acquired illnesses. Candidemia, or bloodstream infection with Candida species, ranks among the top five hospital-acquired infections in several countries, posing significant mortality rates and substantial medical costs. The prevalence of invasive systemic infections and septicemia, particularly in immunocompromised patients, has contributed to increased fatality rates. India's geographical and temporal diversity influences the prevalence of different Candida species, with C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei being common pathogens capable of causing severe systemic diseases, especially in immunocompromised individuals [1].

The emergence of multidrug-resistant Candida species, including C. auris, poses challenges in accurate diagnosis and effective treatment, particularly with non-albicans Candida (NAC) species. Resistance to various drugs, including azoles, echinocandins, and amphotericin B, has been reported, emphasizing the need for active surveillance of Candida infections. Echinocandins are now recommended as first-line treatment for Candidemia, regardless of predisposing factors. Non-culture-based diagnostic methods, such as serological tests and molecular techniques targeting specific fungal genes, are increasingly used for the identification of invasive Candidiasis in routine laboratories.

Despite medical advancements, Candidemia still carries a significant mortality rate, necessitating the development and implementation of rapid diagnostic methods. Polymerase chain reaction (PCR) techniques targeting Candida species directly from blood samples offer a promising approach for early detection and treatment initiation, potentially reducing mortality rates. This study aims to assess the prevalence of Candidemia among patients admitted to the Clinical Hematology department by comparing conventional diagnostic methods with molecular techniques, such as PCR, to improve early detection and treatment outcomes [2-5].

METHODS

Study Design

This prospective observational study was conducted in the Department of Microbiology at King George's Medical University, Lucknow, Uttar Pradesh, India. A total of 187 patients with hematological malignancies were enrolled, and blood samples were collected for analysis. The study was conducted from November 2018 to October 2021 and was approved by the Institutional Ethics Committee of King George's Medical University (letter no. 223/Ethics/R. Cell-18) [6].

Inclusion Criteria

- Age ≥ 15 years
- Presence of high-grade fever or febrile neutropenia (<500 neutrophils/mm2) not responding to broad-spectrum antibiotics for 5 days

Exclusion Criteria

- Patients who received chemotherapy
- Patients who had already completed an antifungal prophylaxis course

Blood Sample Collection

Whole blood samples were collected from patients after obtaining informed consent. A volume of 2 ml was collected in EDTA vials for DNA extraction, and 8-10 ml of blood was collected in BACTECTM Mycosis IC/F blood culture bottles for conventional methods (Becton, Dickinson, and Company, Sparks, MD, USA, and Benex Limited, Dun Laoghaire, Ireland).

Culture and Identification

Blood culture bottles were incubated in a BD BACTEC 9120 machine for up to 15 days, and positive samples were cultured on Mackonky agar (Himedia) and Coulambia agar (5% sheep blood agar, BIOMERIUX). Isolates were identified using microscopy, Germ Tube Test, CHROMagar, Corn meal agar (for hyphae formation), and sugar assimilation. Species identification of Candida was confirmed using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF MS, Biotype CA System, Bruker DaltonicK GmbH, Germany). All Candida isolates were stored at -80°C in 50% glycerol until further use [7].

Antifungal Susceptibility Testing

Antifungal susceptibility testing was performed for voriconazole, amphotericin B, fluconazole, and caspofungin using the Clinical Laboratory Standards Institute (CLSI) guidelines (M27-A3). Sensitivity testing was also conducted using Vitek® MS (bioMerieux Inc.).

Serological Test

Serological testing for Mannan antigen (Mn Ag) was performed using the PlateliaTM Candida Ag Plus immunoenzymatic

assay kit (Bio Rad, Marnes-la-Coquette, France), with results interpreted according to the manufacturer's instructions.

Molecular Methods for Diagnosis of Candidemia

DNA extraction was performed from whole blood samples collected in EDTA vials using the Qiagen DNA extraction kit. DNA concentration was measured using a Bio Spectrometer (Eppendorf, Germany), and extracted DNA was stored at -20°C. Polymerase chain reaction (PCR) was performed targeting the D1/D2 region of 28S rDNA of the large ribosomal subunit and the P-450 lanosterol 14α-demethylase gene specific for Candida species. PCR products were visualized using agarose gel electrophoresis.

Statistical Analysis

Data analysis was performed using the chi-square test of independence to assess associations between variables. Statistical significance was set at $p< 0.05$, and IBM SPSS Statistics software version 21 was used for analysis.

RESULTS

A total of 229 patients with suspected Candidemia and various hematological malignancies were included in this study. Among them, 152 were male and 77 were female, with ages ranging from 10 to 77 years. The mean age of the study population was 33.65 (±16.40) years, and the mean neutrophil count was 4.85 (±4.19). All patients were treated with broad-spectrum antibiotics for more than 72 hours without responding to treatment [8].

During the study period from November 2018 to April 2022, five patients (2.1%) tested positive for Candidemia. Acute Myeloid Leukemia (AML) was the most common hematological malignancy among the patients, accounting for 52.8% of cases, followed by Acute Lymphoblastic Leukemia (ALL) at 24.8%. Symptoms such as cough, chest pain, weakness, body ache, dyspnea, vomiting, and hemoptysis were observed in patients with hematological malignancies (Figures 1-3).

Of the 229 patients, five (2.1%) tested positive for Candidemia via blood culture, while eleven (4.8%) were positive by polymerase chain reaction (PCR), including five patients who were also positive via blood culture. Additionally, 13 (5.6%) serum samples showed positive results for Mannan antigen (Mn Ag) levels. The most common Candida species isolated from blood cultures was C. tropicalis (2.1%), followed by C. auris (0.5%). Antifungal susceptibility testing revealed varying sensitivity patterns for different drugs, with C. tropicalis showing sensitivity to voriconazole (80%) and amphotericin B (80%), while C. auris demonstrated resistance to amphotericin B (20%) and fluconazole (20%) [9].

PCR and Mn Ag demonstrated high sensitivity (100%) and specificity (97.3% and 96.4%, respectively) for diagnosing Candidemia. The combination of PCR and Mn Ag yielded a sensitivity of 84.6%. Conventional methods for Candidemia diagnosis were accurate but time-consuming, requiring expert interpretation and taking a minimum of 5-7 days after positive blood culture results. Automated and molecular methods,

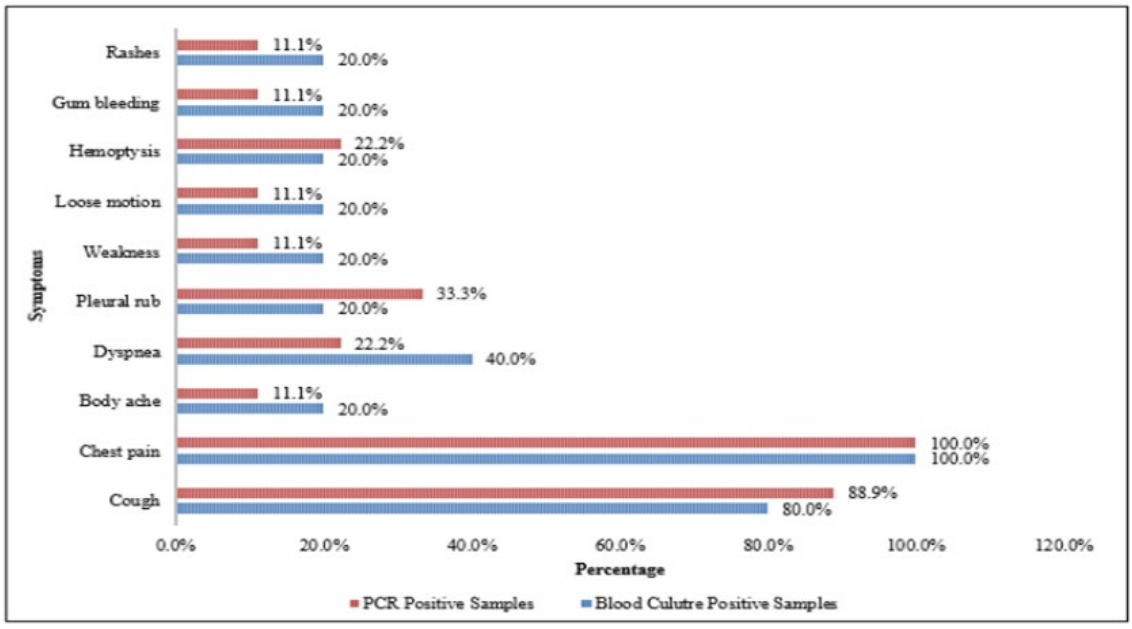

Figure 1: Clinicalf Features and association with Candidemia.

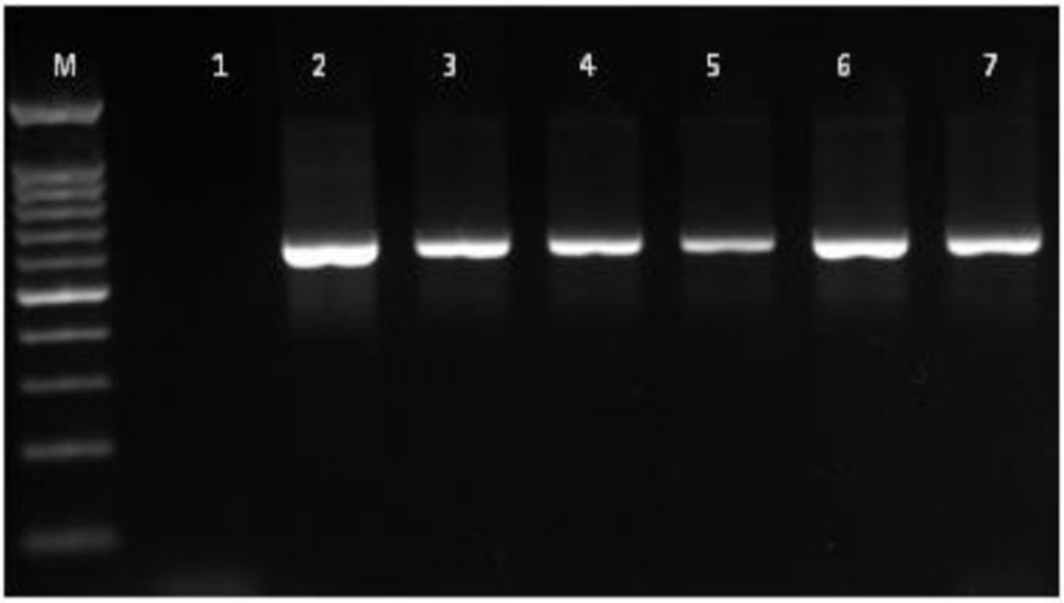

Figure 2: Lane M showing 100 bp DNA Ladder, lane 1 as a negative control, lane 2 showing 600 bp amplified PCR product of fungus DNA, Lane 3,4,5,6,7 showing 600 bp amplified PCR product of fungal DNA.

Test	Sensitivity	Specificity	PPV	NPV
Blood culture Mn Ag assay	38.46	100	100	96.43
Blood Culture and PCR	45.45	100	100	97.32
PCR and Mannan Antigen Assay	84.62	100	100	99.08

Table 1: Sensitivity, specificity, positive predictive value and negative predictive values of blood culture, mannan Ag assay and conventional PCR in combination for the diagnosis of IFIs.

including MALDI-TOF and PCR, offered faster results, with PCR being the most rapid. There was a significant difference in the turnaround time between conventional, automated, and molecular diagnostic methods.

The study findings underscore the importance of rapid and accurate diagnostic methods, such as PCR and Mn Ag testing, in identifying Candidemia among patients with hematological malignancies, allowing for timely and effective infection management (Table 1).

DISCUSSION AND CONCLUSION

The present study underscores the critical role of timely and accurate diagnosis in managing Candidemia, particularly among immunocompromised patients. Candidemia ranks as the fourth most common bloodstream infection in hospitals, posing a significant threat to patient health. Early initiation of appropriate antifungal therapy is crucial for controlling invasive Candida infections, necessitating rapid and precise diagnostic methods to improve outcomes [10].

Our study aimed to compare diagnostic techniques for identifying Candidemia, with a focus on minimizing turnaround time and achieving species-specific identification. Non-culture methods, such as Mn Ag and PCR, offer faster results compared to conventional blood culture or other automated methods. We evaluated the performance of Mn Ag and PCR against blood culture, considered the gold standard for Candidemia diagnosis. Previous research has indicated an increase in diagnostic specificity with Ag testing during Candida infection.

In our study, Candidemia was detected in 2.1% of blood culture samples from suspected cases over a three-year period, with C. tropicalis (2.1%) and C. auris (0.5%) being the predominant isolates among hematological patients. Our

observed prevalence of Candidemia aligns with varying rates reported in previous studies, ranging from 1.6% to 22.9% among hematological patients [11-13,]. Notably, C. tropicalis emerged as the most common cause of Candidemia in our cohort [16-18]. Antifungal susceptibility testing revealed varying sensitivity patterns, with caspofungin being identified as an ideal drug in minimum concentration for treating patients undergoing chemotherapy.

Mn Ag testing showed positive results in a subset of patients, with varying threshold values. Similar findings regarding Ag testing thresholds have been reported in other studies, emphasizing the need for careful interpretation of results based on threshold settings. Additionally, the accuracy of species identification using Maldi-TOF MS was confirmed to be 100%.

Our study highlights the superior effectiveness of conventional PCR over blood culture methods in mycology laboratories. PCR offers rapid and accurate diagnosis, essential for guiding timely therapeutic interventions. Overall, the findings underscore the importance of leveraging advanced diagnostic techniques, such as PCR and Mn Ag testing, to enhance the management of Candidemia in immunocompromised patients.

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