Original Article

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Etiologic Agents of Candidemia in Pediatric Immunocompromised Patients

Sahar Kafshdooz Jabari MSc[1], Mostafa Chadeganipour MD[2], Mohammad Ghahri MD[3], Rasoul Mohammadi MD[4*]

1. Medical Mycology, Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Professor, Medical Mycology, Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Assistant professor, Medical Mycology, Department of Biology, School of Applied Sciences, Imam Hossein University, Tehran, Iran
4. Assistant professor, Medical Mycology, Department of Medical Parasitology and Mycology, Infectious Diseases and Tropical Medicine Research Center, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

*Corresponding author: Dr. RasoulMohammadi, Assistant Professor, Department of Medical Parasitology and Mycology, Infectious Diseases and Tropical Medicine Research Center, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Email: Dr.rasoul_mohammadi@yahoo.com

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Abstract

Background: Candidemia is the main cause of fungal nosocomial bloodstream infections and is related to meaningful mortality specially, in pediatrics. Mortality rate range from 5% to 71%, and it can reach as high as 81%. Delays in beginning of treatment have also been linked to intensified mortality. The epidemiology of Candida infection is changing from region to region. Regional surveillance of the epidemiology of candidemia is necessary to identify patients at highest risk. The aim of this study is rapid and precise detection of Candida species isolated from blood stream by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Materials and Methods: This cross sectional study was conducted during October 2013 to January 2015. Sixteen Candida strains were isolated from 36 patients with positive blood culture in Milad hospital, Resalat Lab., Tehran, Iran. All isolates were identified by PCR-RFLP patterns after digestion with the restriction enzyme HpaII.

Results: Candida albicans (72.2%) and Candida glabrata (22.2%) were the most prevalent species among isolates. Male to female ratio was 9/7, ranging in age from 4-16 years.

Conclusion: Candida albicans remains the most frequently isolated species in the present study; however non-albicans Candida species are increasing. Precise identification of Candida spp. can lead to a better management of candidemia.

Key Words: Candida albicans, Candidemia, Pediatrics, Cancer

Introduction

Candidemia is a main cause of morbidity and mortality in patients with bloodstream infections (BSIs) (1,2). Over the past two decades, the incidence of candidemia has increased significantly (3). Candida species are the most common cause of invasive fungal infections (IFIs) among cancer patients and patients who hospitalized in the intensive care unit (ICU) (2). Invasive procedures, transplantation, immunosuppressive gents, intravenous catheters, and parenteral hyperalimentation are the major risk factors for candidemia(4, 5). The epidemiology of candidemia is changing throughout the world with an increase in the percentage of non-albicans Candida species (6, 7). In order to apply an impressive medication, it is necessary to have an updated knowledge of the proportion of Candida species causing candidemia.

Due to the varying susceptibilities to routinely used antifungal agents such as fluconazole (8), early and precise identification to the species level would help the clinician for more successful management of infection. Non-culture-based techniques like DNA detection by PCR, and PCR-RFLP have been used in order to help the rapid detection of infections (9, 10). Since there are a few studies in this field in Iran, we identified Candida spp isolated from blood stream by PCR-RFLP method with an aim to make better management of candidemia.

Materials and Methods

During October 2013 to January 2015, 850 patients referred to the Milad hospital, ResalatLab.,Tehran, Iran, for a blood culture. Demographic characteristics of patients including gender, age, and residence were registered. In this cross-sectional study the inclusion criteria were as follows: the patients who were resistant to antimicrobial treatments or individuals with any clinical manifestations for candidemia or suppression of immune systems. A total of 36 out of 850 had positive blood cultures (20 cases of bacteria and 16Candida species). A diagnosis of candidemia was made on the basis of ≥1 blood cultures growing Candida species according to the standard practice by using of the automated blood culture system BACTEC 9050 (Becton Dickinson, Sparks, MD, USA). Identification system of Crystal BD and the existence of pertinent clinical manifestations (as listed in the guidelines of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group) was used in the present investigation (11). A positive blood culture with yeasts was sub cultured onto the sabouraud glucose agar (Difco, Detroit, MI, USA), and CHROMagar Candida (Paris, France). Genomic DNA of all isolates was extracted using FTA ® Elute MicroCards (Whatman Inc., Clifton, NJ, USA)(12) following the manufacturer's instructions. Briefly, a loopful of a single colony was suspended in 80-100 µl of distilled water and 5 µl of the suspension was transferred to a disc of FTA card (4 mm in diameter) and incubated at 25°C for at least 5 h. The dried papers were eluted in 400 µl sterile water for 10 seconds, then the paper was transferred to a new microtube containing 40 µl distilled water and incubated at 95 ° C for 15 min. The paper discs were removed and the water including DNA was used for PCR and stored at - 20 °C. Identification of Candida spp. was performed by using of already delineated polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) profiles (10, 13, 14). Briefly, the ITS1-5.8SrDNA-ITS2 region was amplified using PCR mixture including 5µl of 10 × reaction buffer, 0.4 mMdNTPs, 1.5 mM MgCl2, 2.5 U of Taq polymerase, 30 pmol of both ITS1 (5′ -TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′ -TCC TCC GCT TAT TGA TAT GC-3′) primers (14) and 2µl of extracted DNA in a final volume of 50µl. The PCR cycling conditions comprised: initial denaturation at 94 ° C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 ° C for 1 min, with a final extension at 72 °C for 7 min. During the second step, PCR products were digested with the restriction enzyme HpaII (Fermentas, Vilnius, Lithuania). Five microliters of each PCR amplicons and 10µl of RFLP products were separated by gel electrophoresis on 1.5 and 2% agarose gel (containing 0.5 µg/ml ethidium bromide), respectively.

Results

Sixteen Candida strains were isolated from blood culture of total 850 suspected patients referred to a referral hospital in Tehran, Iran, during 15 months. Nine patients (56.2%) were male and seven patients (43.7%) were female, ranging in age from 4 to 16 years. Cancer and ileus were the most predisposing factors among

patients (Table I). Hospitalization period for the patients was 5-47 days in the present study. Seven patients (43.7%) were hospitalized in ICU, 6 patients (37.5%) in cancer ward, 2 patients (12.5%) in general medicine ward, and 1 case (6.2%) in transplantation ward. Most patients had formerly been exposed to broad-spectrum antibiotics (n = 14, 87.5%). Septic shock presented in 2 (12.5%) patients. Candida albicans was the most prevalent species isolated from candidemia (68.7%), followed by Candidaglabrata (25%) (Figure 1, Table II). Colony features on CHROMagar Candida confirmed our findings, as Candidaalbicans, Candidaparapsilosis, and Candidaglabrata isolates gave distinctive green, white, and pink colonies, respectively. Non-albicans Candida species accounted for 31.2% of the cases. Candidaalbicans was the most prevalent species associated with ICU ward (88.8%), and C. glabrata was most repeatedly associated with GI tract disease (75%). Four out of 16 patients (25%) were expired due to the candidemia and immunodeficiency disorders. In two patients that passed away; the etiologic agent of candidemia was Candida albicans. Fourteen patients (87.5%) had non-specific symptoms including fever, chills, pain, nausea, vomiting, and 2 (12.5%) cases were asymptomatic.

Table I. Predisposing factors among patients with candidemia

Predisposing factor	Sex ratio (male to female)	Total number
Cancer	4/3	7 (43.7%)
Ileus	2/2	4 (25%)
Diabetes	1/1	2 (12.5%)
Cerebral tumor	0/1	1 (6.2%)
Heart failure	1/0	1 (6.2%)
Kidney transplantation	1/0	1 (6.2%)

Table II. Candida species isolated from patients with candidemia

Candida spp.	Sex ratio (male to female)	Total number
C. albicans	6/5	11 (68.7%)
C. glabrata	2/2	4 (25%)
C. parapsilosis	1/0	1 (6.2%)

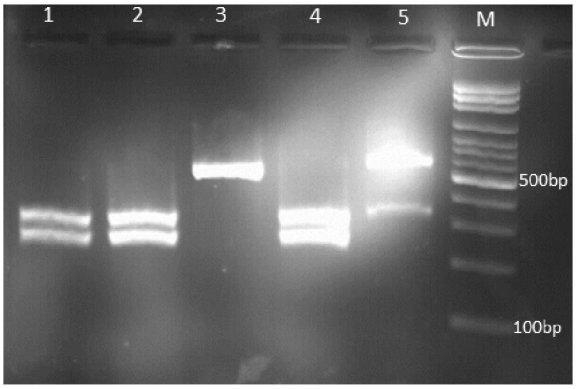

Figure1. Agarose gel electrophoresis of ITS-PCR products of various *Candida* species after digestion with *Hpa*II shows *C. albicans*in lanes 1,2,4,*C. parapsilosis* and *C. glabrata*in lane 3 and 5, respectively, and lane M reveals100 bp DNA size marker

Discussion

Candidemia has been identified among the most prevalent etiologic agents of bloodstream infections. It classified fourth in the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) surveillance study of bloodstream infections in hospitalized patients in the United States (2) and seventh in a national survey of 17 hospitals in Switzerland (15). Candidemia is associated with considerable morbidity and mortality in hospitalized patients. Although Candidaalbicans is the most prevalent species, many reports have noted an increase in the incidence of non-albicans Candida species (3, 16, 17).In an international surveillance project (1997–2003) that contained 134,715 sequential clinical strains of Candida species from 127 medical centers in 39 countries, a trend toward a reduction in Candidaalbicansand an increase in Candidaparapsilosisand Candida tropicalis was noted (7). Moreover, species distribution differences have been described worldwide. For example, Candidaalbicansand Candidaglabratawere most often identified in series from the United States and Denmark, while South America had reduced rates of these Candida species (18). Epidemiology of candidemia varies noticeably from region to region (7). Detection of hematogenous candidiasis or candidemia has been problematic because of the low positivity of blood cultures (19). Even in patients with autopsy-proven systemic candidiasis, the rate of colony growth from blood cultures ranged between 40-60% (20). In spite of the fact that different laboratory tests based on diagnosis of antigens, Candida-specific antibodies, or metabolites have been developed, they all suffer from lack of sensitivity and/or specificity, in addition to being time-consuming (21). Furthermore, these examinations fail to distinctly discriminate between the infecting Candida species.Information that is critical for initiating specific antifungal therapy since several non-albicans Candida species are known to be intrinsically less susceptible to regularly used antifungal agents (22). In order to conquer the limitations of traditional diagnostic tests, DNA-based techniques have been developed for the identification of Candida species and offer a possibly more sensitive means of diagnosing systemic candidiasis. The use of PCR-based tests to detect Candida DNA in body fluids like blood has produced favorable results (21, 23), because more than half of candidemia cases involve, 1 cell/mL, as shown by a study analyzing candidemia cases from 1987 through 1991 (24), and this is difficult to detect this amount of fungi in bloodstream by conventional methods. Although Candidaalbicans was the main Candida species in the present study, the number of non-albicansCandida species, especially Candidaglabrata, was higher than reported onefrom other studies (25, 26). Many investigationsshow 0.2 to 0.5/1000 admissions for candidemia (27, 28), but we found 1.9 patients per 1000 admission in the present study that seems to be overestimated. Mortality rate among patients withcandidemia has been shown to be meaningful, ranging from 14%to 49% (28-31). In agreement with the present study, Candida albicanshas been reported by other researchers to be connected to a high mortality (25, 30). An important limitation of the present study was lack of antifungal susceptibility testing of clinical isolates. A delay in initiation of antifungal therapy with fluconazole among hospitalized patients with candidemia has also been shown to result in a considerably high mortality rate (32, 33). In agreement with these reports, all patients who passed awaydid not take fluconazole in initial stage of candidemia. We mentioned a connection between septic shock and mortality. Two out of four patients who died (50%) revealed septic shock. Aghili et al., (2015) reported

the first case of C. membranaefaciens in Iran, which occurred in a 70-year-old woman, who had coronary artery bypass grafting(34). They isolated germ-tube negative yeast from both blood culture and central venous catheter (CVC) tip culture. They confirmed the results by sequence analysis of internal transcribed spacer region of rDNA. After the removal of the CVC and initiation of fluconazole therapy, the patient was gradually improved and discharged from the hospital. In 2009, Shahhosseiny et al.,(2010), isolated 14 Candida spp. from 2516 blood culture samples (0.5%), and reported Candidaalbicans as the most prevalent species(35).Ghahri et al.,(2012) identified 48 clinical isolates of Candida species from blood specimen cultures of immunocompromised patients by PCR-RFLP method (36). They reported Candidaparapsilosis as the most frequent agent of candidemia, whereas Candidaparapsilosiswas the least frequent species in the present investigation. In 2013, Kalantaret al.,(2013) isolated 5 Candida spp. from 68 blood samples (7.3%) (37). Ahmad et al.,(2002) used the species-specific primers for species-specific identification of Candida species including Candidaalbicans, Candidatropicalis, Candidaparapsilosis, and Candidaglabrata(20). Similar to this study, Horn et al.,(2009) showed that Candidaalbicans was the most common Candida strain in their investigation (45.6%) followed by Candida glabrata(26.0%), Candida parapsilosis(15.7%), Candida tropicalis(8.1%), and Candida krusei(2.5%)(3).However, unlike the findings of the present study, they reported that non–albicans Candida species were more commonly isolated from blood cultures (54.4%).

Conclusion

Candidemia is an important cause of morbidity and mortality among patients infected to fungal infections. The epidemiology of candidemia is altering however in this survey Candidaalbicans was the most common cause of candidemia. Cancer and ileus are significantly associated with candidemia in the present study that highlighting the need for rapid and precise initiation of antifungal therapy in these groups. We emphasize the regional surveillance of the epidemiology of candidemia to identify patients at highest risk like pediatrics and the pattern of causative agents of candidemia in order to develop guidelines for better management of this fatal infection.

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Conflicts of interest

The authors report no conflicts of interest.

References

1. Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. Med Mycol 2007; 45:321-46.
2.Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004; 39:309-17.
3.Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin infectious dis 2009; 48:1695-703.
4.Wisplinghoff H, Seifert H, Tallent SM, Bischoff T, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features

and susceptibilities. Pediatr Infect Dis 2003; 22:686-91.

5.Peres-Bota D, Rodriguez-Villalobos H, Dimopoulos G, Melot C, Vincent JL. Potential risk factors for infection with Candida spp. in critically ill patients. Clin Microbiol Infect 2004; 10:550-5.

6.Antoniadou A, Torres HA, Lewis RE, Thornby J, Bodey GP, Tarrand JP, et al. Candidemia in a tertiary care cancer center: in vitro susceptibility and its association with outcome of initial antifungal therapy. Medicine 2003;82:309-21.

7. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans Candida spp among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis 2010; 14:e954-e66.

8. Zilberberg MD, Kollef MH, Arnold H, Labelle A, Micek ST, Kothari S, et al. Inappropriate empiric antifungal therapy for candidemia in the ICU and hospital resource utilization: a retrospective cohort study. BMC infect Dis 2010; 10:150.

9.McMullan R, Metwally L, Coyle P, Hedderwick S, McCloskey B, O'Neill H, et al. A prospective clinical trial of a real-time polymerase chain reaction assay for the diagnosis of candidemia in nonneutropenic, critically ill adults. Clin Infect Dis 2008; 46:890-6.

10.Mohammadi R, Mirhendi H, Rezaei-Matehkolaei A, Ghahri M, Shidfar MR, Jalalizand N, et al. Molecular identification and distribution profile of Candida species isolated from Iranian patients. Med Mycol 2013; 51:657-63.

11. Ascioglu S, Rex J, De Pauw B, Bennett J, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002; 34:7-14.

12. Borman AM, Linton CJ, Miles S-J, Campbell CK, Johnson EM. Ultra-rapid preparation of total genomic DNA from isolates of yeast and mould using Whatman FTA filter paper technology-a reusable DNA archiving system. Med Mycol 2006;44:389-98.

13.Mohammadi R, Nazeri M, Mesdaghinia E, Mirhendi SH. Identification of Candida Species among Patients with Vulvovaginal Candidiasis in Kashan by PCR-RFLP Method. J Isfahan Med Sch 2012; 29(165):1-8.

14. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. Nippon Ishinkin Gakkai Zasshi 2006; 47:225-9.

15. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin Infect Dis 2004; 38:311-20.

16.Sipsas NV, Lewis RE, Tarrand J, Hachem R, Rolston KV, Raad II, et al. Candidemia in patients with hematologic malignancies in the era of new antifungal agents (2001-2007). Cancer 2009; 115:4745-52.

17. Krcmery V, Barnes A. Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect 2002;50:243-60.

18. Pfaller M, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 2007; 20:133-63.

19. Groll A, Ritter J. Diagnosis and management of fungal infections and pneumocystis pneumonitis in pediatric cancer patient. Klinische Padiatrie 2005; 217:S37-66.

20. Ahmad S, Khan Z, Mustafa AS, Khan ZU. Seminested PCR for diagnosis of candidemia: comparison with culture, antigen detection, and biochemical methods for species identification. J Clin Microbiol 2002; 40:2483-9.

21. Ellepola A, Morrison CJ. Laboratory diagnosis of invasive candidiasis. J Microbiol 2005; 43:65-84.

22.Posteraro B, Sanguinetti M, Masucci L, Romano L, Morace G, Fadda G. Reverse cross blot hybridization assay for rapid detection of PCR-amplified DNA from Candida species, Cryptococcus neoformans, and Saccharomyces cerevisiae in clinical samples. J Clin Microbiol 2000; 38:1609-14.
23. Khan Z, Mustafa A. Detection of Candida species by polymerase chain reaction (PCR) in blood samples of experimentally infected mice and patients with suspected candidemia. Microbiol Res 2001; 156:95-102.
24.Pfeiffer CD, Samsa GP, Schell WA, Reller LB, Perfect JR, Alexander BD. Quantitation of Candida CFU in initial positive blood cultures. J Clin Microbiol 2011; 49:2879-83.
25. Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen RA, Powderly W, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis 2003;37:634-43.
26.Tortorano AM, Kibbler C, Peman J, Bernhardt H, Klingspor L, Grillot R. Candidaemia in Europe: epidemiology and resistance. Inter J Antimicrob Agen 2006;27:359-66.
27.Kibbler C, Seaton S, Barnes RA, Gransden W, Holliman R, Johnson E, et al. Management and outcome of bloodstream infections due to Candida species in England and Wales. J Hosp Infect 2003; 54:18-24.
28. Zaoutis TE, Argon J, Chu J, Berlin JA, Walsh TJ, Feudtner C. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin Infect Dis 2005; 41:1232-9.
29. Gudlaugsson O, Gillespie S, Lee K, Berg JV, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis 2003; 37:1172-7.
30.Weinberger M, Leibovici L, Perez S, Samra Z, Ostfeld I, Levi I, et al. Characteristics of candidaemia with Candida-albicans compared with non-albicans Candida species and predictors of mortality. J Hosp Infect 2005; 61:146-54.
31. Malani A, Hmoud J, Chiu L, Carver PL, Bielaczyc A, Kauffman CA. Candida glabrata fungemia: experience in a tertiary care center. Clin Infect Dis 2005; 41:975-81.
32. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis 2006; 43:25-31.
33. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agen Chemoth 2005; 49:3640-5.
34.Aghili SR, Shokohi T, Boroumand MA, Fesharaki SH, Salmanian B. Intravenous Catheter-Associated Candidemia due to Candida membranaefaciens: The First Iranian Case. J Tehran Heart Cent 2015; 10:101-5.
35.Shahhosseiny MH, Nematian Soteh M, Ghahri M, Saadatmand S, Hosseiny SA. Identification of CandidaSpecies by Seminested PCR in Candidemia. Iranian J Med Microbiol 2010;4:91-9.
36.Ghahri M, Mirhendi H, Imani FAA, Beyraghi S. Species Identification of Candida Strains Isolated from Patients with Candidemia, Hospitalized in Tehran, by Enzymatic Digestion of ITS–rDNA. Isfahan Med Sch 2012.29.
37.Kalantar E, Assadi M, Pormazaheri H, Hatami S, Barari MA, Asgari E, et al. Candida non albicans with a High Amphotericin B Resistance Pattern Causing Candidemia among Cancer Patients. Asian Pacific journal of cancer prevention: APJCP 2013; 15:10933-5.