RESEARCH ARTICLE

Candida non albicans with a High Amphotericin B Resistance Pattern Causing Candidemia among Cancer Patients

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Abstract

Background: Many scientists have reported *Candida species* to be of great concern because of the high frequency that they colonize and infect human hosts, particularly cancer patients. Moreover, in the last decades *Candida* species have developed resistance to many antifungal agents. Based on this, we aimed to identify and determine the prevalence of *Candida spp* from blood culture bottles among cancer patients and their antifungal resistance pattern. Materials and Methods: From the blood culture bottles isolation and identification of the *Candida spp* were performed by conventional microbiological techniques. The *in vitro* antibiotic resistance pattern of the isolates was determined by CLSI guidelines. Genomic DNA was isolated and amplified. Each gene was separated by agar gel electrophoresis. Results: Identification of *Candida* spp was based on the presence of yeast cells in direct examination, culture and DNA extraction. Of the 68 blood samples collected during the study period (April 2013 to October 2013), five (7.35%) were positive for the presence of *Candida spp*, 2 (40%) of which were identified as *Candida albicans* and 3 (60%) were *Candida non-albicans*. Conclusions: High resistance to amphotricin B was observed among all the *Candida non-albicans* isolates. Regular investigations into antifungal resistance will help us to get an updated knowledge about their antibiotic resistance pattern which may help the physician in selecting the antibiotics for empirical therapy.

Keywords: Candidemia - cancer patients - *Candida non albicans* - multiplex PCR - amphotericin B resistance

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Introduction

Cancer patients continue at substantial risk for developing serious infections like candidemia despite considerable advances in cancer therapy. Candidemia is a serious problem that needs immediate attention and management. It is a cause of high mortality especially if caused by resistant *Candida* spp (Hadley et al., 2002; Iranparast et al., 2014).

Among the fungi of medical importance, *Candida* spp are of great concern because of the high occurrence that they infect human hosts particularly cancer patients. Among the 20 species of *Candida* of medical interest, *Candida albicans* is the most prevalent yeast can develop infection and disease (Colomboa et al., 2013). As cancer is the third cause of death in Iran (Mousavi et al., 2009); and also many scientists reported (Edmond et al., 1999; Theoklis et al., 2005) candidemia among this kind of patients has increased markedly in hospitalized patients and based on our knowledge scanty information is available on non-albicans *Candida* causing candidemia and its antifungal resistance pattern in Iran therefore we aimed to identify and determine the prevalence of *Candida* spp from blood culture bottles among cancer patients and their antifungal resistance pattern to get an updated knowledge about their antibiotic susceptibility pattern which may help the physician in selecting the antibiotics for empirical therapy.

Materials and Methods

The cancer research hospital is a 240 bed having medical/oncology/chemotherapy/radiotherapy/surgical intensive care units which is the major hospital of the National Cancer Institute, Tehran, Iran and affiliated to Tehran University of Medical Sciences. Similarly, Kasra Hospital is a 70 bed having medical/surgical/intensive care units/CCU/Post CCU/NICU/emergency and Labour which is one of the major private hospital of the Alborz province, Karaj, Iran.

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A single blood culture was taken and inoculated into one blood culture bottle; the inoculated bottle was inspected daily; if bottle showed growth, the blood culture result was considered positive. After isolation of the pure colony, further biochemical identifications were done by conventional methods. MIC of antifungal susceptibility patterns for each isolate was determined by broth dilution (CLSI, 2010). The *Candida* spp were tested against the following antifungal commonly used at the both centers; amphotricin B, nystatin, ketokenazole and fluconazole. This study was also approved by the Ethics Committee of Alborz University of Medical Sciences, Karaj, Iran.

DNA extraction & Multiplex-PCR

Candida strains cultured overnight at 37°C in SDA medium. Genomic DNA was isolated by DNA extraction according reference protocol (Ligozzi et al., 2003). PCR amplification of target DNA was done in a total volume of 50µl. The reaction mixture contained 5µl 10×amplification buffer [500mM KCl, 100mM Tris/HCl (pH 8.5), 1.0% Triton X-100], 1µl 25mM MgCl2, 0.6µl each of 2.5mM dNTPs (Fermentas, GmbH, Germany), 1µl forward and reverse primers for all genes (20ng/µl), 0.4µl Taq DNA polymerase (5U/µl), and 80 pg extracted DNA. PCR conditions were; initial denaturation at 94°C for 4 min, followed by 32 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 5 min. Multiplex PCR was carried out by the addition of primer pairs for 18S ribosomal RNA gene in the reaction mixture. Primer sequences used in this study are shown in Table 1. We designed primers by Alell ID 6 software. Amplified products were identified by agarose (1.5%) gel electrophoresis in 1×TBE, and stained by ethidium bromide.

Results

Identification of *Candida* spp was based on the presence of yeast cells in direct examination, culture and DNA extraction. Of the 68 blood samples collected during the study period (April

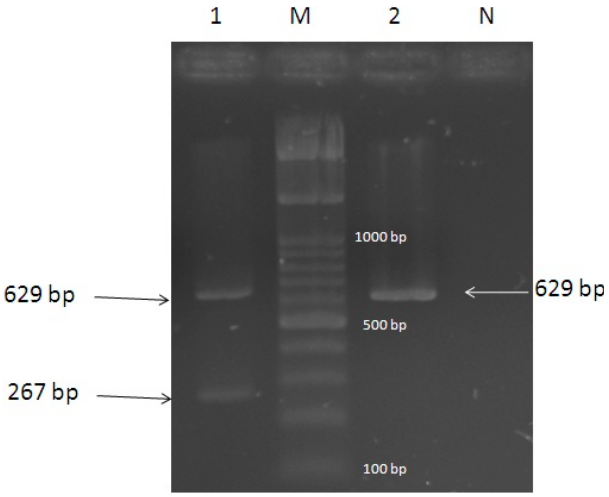
2013 to October 2013), five (07.35%) were positive for the presence of *Candida* spp, 02 (40%) of which were identified as *Candida albicans* and 03 (60%) were

Table 1. Primers Used in this Study

Primers	Sequence (5'à3')	Reference
CA-f	CAACTTGTCACACCAGATTATTAC	This study
CA-r	TCCCGCCTTACCACTACC	This study
CN-f	CCGATTGAATGGCTTAGTG	This study
CN-r	CCTCCGCTTATTGATATGC	This study

Table 2. Determination of MIC for Candida Spp Caused Candidemia Among Cancer Patients

Antibiotic	C. albicans (03)	C. non - albicans (02)
	µg/ml	
Amphotricin B	1.5	6
Nystatin	2	4
Ketoconazole	1	4
Fluconazole	1	0.5



ure 1. Agarose Gel Electrophoresis of the Genomic DNA of Candida Isolates from Cancer Patients: Lane 1 *Candida albicans*, Lane 2 Marker, Lane 3 Candida spp., Lane 4 Negative Control

Candida non-albicans. As shown in Table 2 all the three *Candida non-albicans* isolates showed high resistance pattern to amphotericin B (MIC 6.0Ig/mL); however, C. albican isolates were relatively lees resistance pattern (MIC1.5 Ig/ml). The identity of all the *Candida* sp. and *Candida albicans* was confirmed 629 and 267 bp length of products by multiplex PCR, respectively. (Figure 1).

Discussion

Although a variety of drugs are now available for the treatment of cancer but in Iran cancer related deaths accounted for 30000 of the 300000 deaths in 2003 (Naghavi, 2003). Usually cancer patients experience a high incidence of a variety of infections particularly blood stream infections and eventually many of these patients die due to infection (Blumberg et al., 2013; Hajjeh et al., 2004; Fridkin et al., 2005). This study demonstrated that *C. non - albicans* was the predominant species of Candidemia in patients with cancer which is in agreement with other reports from Iran (Badiee et al., 2011; Badiee et al., 2009). These isolates showed high amphotricin resistance pattern which is similar Frederick et al. observation and also Badiee et al. study from Iran. (Badiee et al., 2011; Nolte et al., 1997)

As other study from Iran observed and believed that the great importance of *non -albicans Candida* as a pathogen in clinical samples (Badiee et al., 2009); we also conclude that the remarkable point in our study is high resistance to amphotricin B was observed among all the *Candida non-albicans* isolates. Regular investigations into antifungal resistance will help us to get an updated knowledge about their antibiotic resistance pattern which may help the physician in selecting the antibiotics for empirical therapy. Furthermore, these data indicate that non-albicans *Candida* spp. and their susceptibility patterns must be considered when treating the cancer patients. Last but not the least for detection and identification of Candid species here for the first time in Iran we report, one step multiplex PCR for detection of *Candida* spp.

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