Polymerase Chain Reaction Melting Profiles of *Candida glabrata* Clinical Isolates in Solid Organ Transplant Recipients in Comparison to the Other Group of Surgical Patients

M. Golas[a,*], I. Netsvyetayeva[a], K. Piskorska[a], M. Sikora[b], M. Jaworska-Zaremba[c], E. Mierzwinska-Nastalska[c], L. Paczek[d], and E. Swoboda-Kopec[b]

[a]Department of Medical Microbiology, Warsaw Medical University, Warsaw, Poland; [b]Department of Dentistry Microbiology, Warsaw Medical University, Warsaw, Poland; [c]Department of Prosthetic Dentistry, Warsaw Medical University, Warsaw, Poland; [d]Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

ABSTRACT

The aim of the retrospective study were to estimate the prevalence of *Candida glabrata* in liver and kidney transplant recipients compared to patients with short bowel syndrome receiving chronic total parenteral nutrition and relevance of the polymerase chain reaction melting profile (PCR MP) method for *Candida glabrata* strains differentiation. *C. glabrata* clinical strains isolated from patients were identified by using standard mycological procedures. The analysis of genetic relatedness of the isolated strains was conducted using the PCR MP method. The prevalence of *C. glabrata* comprised 29% of all episodes of fungal colonization and infection in solid organ transplant recipients, and 54% of those in hospitalized patients receiving long-term total parenteral nutrition. Among 78 isolates obtained from 55 solid organ transplant recipients and 2 organ donors, 44 different *C. glabrata* PCR MP fingerprints were observed. Forty-seven organ recipients and one organ donor carried unique *C. glabrata* strains. Among 37 isolates obtained from 31 patients receiving long-term TPN, 8 different PCR MP profiles of *C. glabrata* strains were observed. Two patients carried unique *C. glabrata* strains. Most of the *C. glabrata* colonization and infections in solid-organ transplant recipients were caused by endogenic strains. Most of the *C. glabrata* colonization and infections in hospitalized patients receiving long-term total parenteral nutrition could result by patient-to-patient transmission. The results showed that the PCR MP technique is a good discriminatory method for genotyping for *C. glabrata* strains.

BECAUSE of progress such as advanced surgical techniques, there is frequent use of immunosuppressive therapy and an increasing number of parenterally fed patients. The incidence of *Candida spp.* infections in humans increase continuously. *Candida* invasive infections are characterized by a high mortality rate, as well as by the development of resistance to azole therapy, especially fluconazole [1–3]. In some locations and in the presence of immunosuppressed patients, *Candida spp.* may cause systemic mycosis in patients from high-risk groups, especially those undergoing induction therapy, those with acute myeloid leukemia, myelodysplastic syndrome, receiving chronic total parenteral nutrition, and after undergoing solid organ transplantation [2,3]. The use of antimycotics may promote selection of *Candida* resistance to treatment, especially in prophylaxis and empiric therapy, where resistant strains may be selected. This has a great importance in case of transplant recipients highlighting the therapeutic difficulties of fungal infections [1,4,5].

C. glabrata is one of the most frequently isolated non-*albicans Candida* species. In the past years, *C. glabrata* infections have increased possibly as a result of the wide use of azoles that promote rapid selection of resistance [6–8].

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*Address correspondence to Marlena Golas, Department of Medical Microbiology, Warsaw Medical University, Chalubinskiego Street 5, 02004 Warsaw, Poland. E-mail: marlena.gol@gmail.com

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During the last several years, molecular methods have been widely used in the study of strains relatedness. These methods allowed retrospective examinations of putative candidiasis outbreaks and assessments of epidemiological aspects of such outbreaks. Random amplification of polymorphic DNA (RAPD) was often used to fingerprint strains and for determination of the potential infection source [9–12]. One of the latest modifications of the RAPD method is the polymerase chain reaction (PCR) melting profile (MP) method developed by Masny and Pluciennicczak that has at least the same discriminatory power as RAPD. It is inexpensive, simple, and gives results in a short time [13,14].

Molecular tests used in epidemiological analysis of candidiasis may help to improve medical procedures and patients' security by establishing procedures to reduce nosocomial infections [10].

The aim of the retrospective study were to estimate the prevalence of *Candida glabrata* in liver and kidney transplant recipients in comparison to patients with short bowel syndrome receiving chronic total parenteral nutrition and relevance of the PCR MP method for *Candida glabrata* strains differentiation in both groups.

MATERIALS AND METHODS

Study Population

Materials for the analysis consisted of catalogued *Candida glabrata* strains cultured from clinical materials obtained from 35 kidney and 20 liver transplant recipients and 31 patients with short bowel syndrome secondary to bowel resection receiving long-term total parenteral nutrition (TPN).

Antifungal Prophylaxis in Study Population

Kidney transplant recipients had no antifungal prophylaxis after the transplantation (n = 35 patients).

Liver transplant recipients received fluconazole prophylaxis for 1 to 3 months after transplantation (400 mg/d; n = 20 patients).

Patients receiving TPN had no systemic antimicrobial prophylaxis (n = 17 patients). Only TPN patients who have a history of multiple catheter-related blood stream infections received 200 mg fluconazole per week in prophylaxis (n = 14 patients).

Strains

Clinical samples were collected through routine mycological diagnostic procedures from patients hospitalized in the Institute of Transplantation Medicine, and in the Department of Surgery and Nutrition, Medical University of Warsaw, in 2010 and 2011. *C. glabrata* was cultured from 78 clinical samples, taken from 55 recipients and two organ donors; they included 34 samples of urine, 13 samples collected from peritoneal cavity, 1 abdominal abscess, 4 tracheal aspirates, 9 pus samples, 2 pharyngeal swabs, 1 bronchovesicular lavage, 5 surgical wound swabs, 3 bile samples, 4 abdominal drains, 1 intravascular devices, and 1 blood sample. Twenty-eight strains were collected from 11 patients who were diagnosed with invasive candidiasis. Invasive candidiasis was defined as isolated yeast-like fungal strains from sterile body sites. Another 50 strains were collected from 46 patients with fungal colonization.

C. glabrata was cultured from 37 clinical samples obtained from 31 patients with short bowel syndrome resulting from bowel resection and receiving long-term TPN; these included 9 samples of urine, 7 anal swabs, 6 stoma fluids, and 9 other materials specimens. There were strains which colonized as well as caused infections in organ recipients and also isolates providing colonization in group of patients receiving chronic TPN.

Identification. Identification was performed with the use of standard reference mycological procedures. Micro-organisms were grown at 30°C on Sabouraud dextrose agar with the addition of chloramphenicol and gentamicin (bioMerieux, Marcy l'Etoile, France). The identification of the *Candida* species was conducted with the use of chromogenic medium CHROMagar *Candida* (Becton Dickinson, Heidelberg, Germany) and biochemical assimilation test ID 32 C (bioMerieux).

DNA isolation. The isolation of genomic DNA was performed according to the manufacturer's protocol enclosed with the bacterial and yeast DNA isolation kit (EURx, Gdansk, Poland). DNA was isolated from 3-day-old yeast cultures in 1.5-mL Sabouraud liquid broth without any addition of antibiotics. After removal of the broth remains, the enzymatic lysis of the cells was performed in the reaction buffer containing proteinase K and ribonuclease. All obtained lysates were applied to the binding spin-columns and washed twice with the buffer for the purpose of lipid and protein removal. The elution of DNA bounded to the membrane was performed using Tris buffer (pH 8.0).

Molecular typing. The genetic relatedness analysis was conducted using the PCR MP method (DNA-Gdansk, Gdansk, Poland), according to the manufacturer. During the first step, the genomic DNA was completely digested by the restriction enzyme *Hind*III. In the next step, the restriction fragments were ligated with an oligonucleotide adapter. Finally, during PCR, DNA fragments were amplified. Reactions of amplification were conducted in the DNA Engine thermocycler (BioRad, Hercules, Calif, United States), in the following conditions 72°C – 1′, 72°C – 1′, 80°C – 1′, 22 cycles: 80°C – 30″, 72°C – 2′15″, final elongation 72°C – 5′. The reaction products were separated in the 2% agarose gels, stained with ethidium bromide, and visualized by illumination with the ultraviolet light. The analysis of the results was performed using the GeneTools program (Syngene, Cambridge, United Kingdom).

RESULTS

Mycological tests revealed the presence of yeast-like fungi in 275 samples obtained from 190 organ transplant recipients and 7 organ donors. *C. glabrata* was found to be second in prevalence (after *C. albicans* 52%), and composed approximately 20% of all episodes of fungal colonization and infection in solid organ transplant recipients.

Mycological tests revealed the presence of yeast-like fungi in 55 samples taken from patients with short bowel syndrome resulting from bowel resection and receiving long-term TPN. *C. glabrata* was the most common fungal pathogen and composed approximately 54% of total positive samples.

Among 37 isolates obtained from 31 patients receiving long-term TPN, 8 different PCR MP patterns of *C. glabrata* strains were observed. Two patients carried strains that were not detected in other patients—they were colonized by unique *C. glabrata* strains. Figure 1 presents the PCR MP electrophoretic separation for *C. glabrata* strains isolated from long-term TPN patients.