ORIGINAL ARTICLE

CANDIDA GLABRATA: AN EMERGING THREAT FOR THE IMMUNOCOMPROMISED

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ABSTRACT

Background: Fungal infections cause a significant amount of morbidity and mortality among immuno compromised population. Various fungal agents are responsible but candida are one the most frequently isolated ones. Recently, a redistribution of candida species has been observed that has highlighted a non Candida albicans species i.e. Candida glabrata. High frequency of Candida glabrata exhibits high resistance rates and it has emerged as a difficult to treat pathogen. The objective of this study was to identify the various strains of fungi and their sensitivity in immuno compromised patients.

Material & Methods: This was a cross-sectional study in which immunocompromised patients were screened for fungal infections on the basis of conventional and API 20-C methods. In this study 165 cases were inducted that included cases on dialysis, chemotherapy for malignancies, those with transplant and receiving immunosuppressive therapy and were positive for fungal infections.

Results: Candida glabrata was isolated from 39(23.6%) cases and of these isolates, 13% were resistant to fluconazole.

Conclusion: Apart from Candida albicans, Candida glabrata is another difficult to treat fungal agent in immunocompromised patients.

KEY WORDS: Candida glabrata, Non albicans candida, Immunocompromised patient.

INTRODUCTION

The incidence of invasive fungal infections has increased considerably as reported in recent studies.[1] Invasive fungal infections are significant cause of morbidity and mortality in patients with hematological malignancies and those who have undergone allogeneic hematopoietic stem cell transplantation. Various fungal agents are responsible for this complication, but Aspergillus and Candida spp. are the most frequently isolated microorganisms; less commonly, infections could be caused by Zygomycetes or other rare molds or yeasts. The increasing diversity of fungal pathogens in high-risk patients, the differences in the antifungal spectra of available agents and the increasing rates of resistance call for identification of the infecting isolate at the species level and for information on drug resistance, in order to provide state-of-the-art patient care. Microscopy and culture of appropriate specimens remain the reference standard for mycological diagnosis, despite difficulties in obtaining appropriate and/or sufficient specimens, long durations of culture and false-negative results. Early recognition and rapid initiation of effective treatment is a prerequisite for successful management of immunocompromised cases with invasive fungal infections.[2]

Candidiasis is most commonly caused by the overgrowth of Candida albicans but it is not the only species that causes infections in humans, whether superficial or systemic. Until recently, Candida glabrata has been considered as a non-pathogenic saprophyte rarely causing any serious infection but recent studies indicate that after Candida albicans the next main cause is Candida glabrata. The symptoms are very similar to those of Candida albicans however the key difference lies in the line of treatment.[3]

Although C. glabrata infection is second or third in frequency to C. albicans, it is difficult to treat, and associated with a high mortality rate in immuno-compromised, at-risk hospitalized patients, but there have been relatively few investigations focused on C. glabrata as compared to other Candida species. Candida glabrata is an increasing cause of candidemia, especially at cancer and bone marrow transplant centers where fluconazole is used for antifungal prophylaxis. This

yeast is less susceptible to fluconazole in vitro than Candida albicans.[4] Fungemia-related mortality of C. glabrata is higher than that for C. albicans.[5]

The objective of this study was to identify the various strains of fungi and their sensitivity in immuno compromised patients.

MATERIAL AND METHODS

This is a cross sectional study that included immuno-compromised subjects. A total of 300 subjects were inducted comprising of 250 immunocompromised cases and 50 healthy controls.

Samples included Swabs from oral and skin lesions, high vaginal swabs, sputum, urine and blood that were collected from the department of Radio-Therapy, Atomic Energy commission, Oncological samples from Department of Paeds Oncology, NICH, National Institute of Blood Diseases & Transplantation Center, Sindh Institute of Urology & Transplantation ,and The Kidney Center. The study was carried out at the department of Microbiology in the Basic Medical Sciences Institute, JPMC & Blood Diseases Center Karachi.

Table 2: Various Clinical samples taken from Immunocompromised patients (n=459)

Type of Samples	Number	Percentage
Oral swabs	212	46.2%
Urine	87	18.9%
High Vaginal swabs	55	11.9%
Skin scraping and Nail clipping	28	6.1%
Sputum	27	5.9%
Tips of I/V Cannula	12	2.6%
Blood	06	1.3%
Chest intubation	06	1.3%
CSF	02	0.4%
Pus from Ocular lesion	01	0.2%
Total	459	100%

Samples were processed by conventional methods that included direct microscopy and culture.

Isolates obtained by culture on blood agar, Sabouraud's Dextrose agar and Corn Meal agar supplemented with Tween 80 and confirmed by germ tube and sugar fermentation tests.Stock cultures of isolates were maintained on Sabouraud's Dextrose agar (SDA) slants at 4 C.

Identification of Candida species was also done by API- 20 Candida method.

Sensitivity to Fluconazole was carried out on Mueller Hinton's Agar supplemented with 2% Glucoseand 0.5 mcg/ml methylene blue.Inoculum was adjusted to match 0.5 McFarland's density standard to obtain a concentration of 1-5 x10 6 yeast cells /ml. Zone diameters were read at 80%growth inhibition as in NCCLS,M27-A reference dilution method.6

RESULTS

Samples were obtained from both male and female cases including paediatric cases and adults (Table 1) and consisted of oral and skin lesions, high vaginal swabs, sputum, urine and blood. (Table 2).

Out of 250 cases, 165 samples showed results of species identification on the basis of conventional and API–Candida methods and 39 (23.6%) were identified as C. glabrata. (Table 3)

Other species identified were C. albicans (51.5%), C. krusei (7.2%).

Resistance to Fluconazole was found in 5 cases of C.glabrata.

Table 1: Distribution of Immunocompromised patients according to Age and Sex Group

Age Group (Years)	Female	Male	Total	Cumulative Frequency
<2	03 (1.00%)	07 (2.33%)	10 (3.33%)	44.7%
2-14	20 (6.67%)	17 (5.67%)	37 (12.34%)	57.0%
15-50	93 (31.00%)	41 (13.67%)	134 (44.67%)	60.3%
>50	73 (24.33%)	46 (15.33%)	119 (39.66%)	100%
Total (%)	189 (63%)	111 (37%)	300 (100%)	

Table 3: Comparison of conventional methods and API-20 Candida for the identification of various species

Name of Species	Conventional Method		API-Candida	
	Number	%age	Number	%age
Candida albicans	85	51.5%	85	51.5%
Candida glabrata	39	23.6%	39	23.6%
Candida tropicalis	13	7.9%	13	7.9%
Candida krusei	12	7.3%	12	7.3%
Candida parapsilosis	–	–	05	3.0%
Candida guilliermondii	–	–	02	1.2%
Candida kefyr	–	–	02	1.2%
Candida stellatoidea	02	1.2%	02	1.2%
Candida famata	–	–	02	1.2%
Saccharomyces cerevisiae	–	–	01	0.6%
Geotrichum species	–	–	01	0.6%
Cryptococcus neoformans	–	–	01	0.6%

Table 4: Percentage of sensitivity to Fluconazole in different species of candida (n=162)

Name of Species	No. of	Sensitivity		Resistance	
	Species	Number	Percentage	Number	%age
Candida albicans	85	78	92%	07	8.0%
Candida glabrata	39	34	87%	05	13.0%
Candida tropicalis	13	10	77%	03	23.0%
Candida krusei	12	06	50%	06	50.0%
Candida parapsilosis	05	04	80%	01	20.0%
Candida guilliermondii	02	02	100%	00	0
Candida kefyr	02	01	50%	01	50.0%
Candida stellatoidea	02	02	100%	00	0
Candida famata	02	02	100%	00	0

Statistical analysis was done and the C.glabrata was found to be identified in a significant no. of cases.

DISCUSSION

Non-albicans candida (NAC) species has been shown to cause 35-65% of all candidaemias in the immunocompromised population. They occur more frequently in cancer patients, mainly with haematological malignancies and bone marrow transplant (BMT) recipients (40-70%), but are less common among intensive care unit (ICU) and surgical patients (35-55%), children (1-35%) or HIV-positive patients (0-33%). There is no difference between overall and attributable mortality, with the exception of C. glabrata which tends to infect immunocompromised individuals. While the crude mortality is low, attributable mortality (fungaemia-associated mortality) is higher than with C. albicans.[7]

A retrospective study was done to compare the epidemiology of candidaemia and its risk factors in patients with hematologic malignancies (HM) with those in patients who had solid tumors (ST). The medical and electronic records of all patients with cancer who had candidaemia at The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA. from 1993 to 2003 were reviewed for demographic data and clinical information, including the use of prophylactic fluconazole, the infecting Candida species, and the source of candidaemia (catheter-related vs other apparent sources). C. glabrata was among the leading causes of candidaemia in patients with HM. Neutropenia was the leading risk factor for all candidemia.[8]

Bloodstream infections (BSIs) caused by Candida glabrata have increased substantially. Candida glabrata often shows resistance to fluconazole therapy. A case control study was conducted at 3 hospitals from January 1, 2003, to May 31, 2007. The 2 case groups included patients with fluconazole-resistant C glabrata BSIs (minimum inhibitory concentration >16 μg/mL) and patients with fluconazole-susceptible C glabrata BSIs (minimum inhibitory concentration < 8 μg/mL) Previous fluconazole use has been found to be a significant risk factor for health care–associated fluconazole-resistant C glabrata BSIs. Further studies are needed to evaluate the effect of decreasing fluconazole use on rates of fluconazole-resistant C glabrata BSIs.[9]

Among the uncommon candida species only *C. glabrata* can be said to be truly emerging as a cause of BSIs, due in part to its intrinsic and acquired resistance to azoles and other commonly used antifungal agents among the *Candida* spp. *C. glabrata* alone has increased in incidence as a cause of BSIs in U.S. intensive care units since 1993. Likewise, in certain regions of the United States *C. glabrata* is a common cause of BSI and is also often resistant to fluconazole.[10,11]

The emergence of *C. glabrata* as an important cause of BSIs is not simply a result of selection of an antimycotic on a routine basis (e.g., fluconazole) but may be influenced by patient age, underlying diseases, geographic location, or other unknown factors.[13] In contrast to other Candida species, C. glabrata is not dimorphic; consequently, it is found as blastoconidia both as a commensal and as a pathogen. C. glabrata infections are difficult to treat and are often resistant to many azole antifungal agents, especially fluconazole.[14.15]

C.glabrata has been established as an important pathogen as far as minimum inhibitory concentrations (MIC) for fluconazole is concerned.

A patient with newly diagnosed yeast infection can face complications if the clinician has reason to believe the patient is at risk of C. albicans infection. Fluconazole, arguably less toxic and several-fold less expensive may need to be withheld in these patients pending yeast identification.[16-18]

Early mycological diagnosis and antifungal therapy are crucial for surviving invasive fungal infections (IFIs) in patients of all ages and in vitro susceptibility testing should be performed for most species of NAC in addition to removal of any foreign body to optimize management.[19-21]

It is strongly recommended that species directed' therapy should be administered for fungaemia according to the species identified-(amphotericin B for C. glabrata, fluconazole for other species, including polyene-resistant or tolerant Candida species e.g C. lusitaniae, C. guilliermondii).

REFERENCES

1 Parisa Badieeab, Parivash Kordbacheha, Abdolvahab Alborzib, SeyedAli Malekhoseinic, Mani Ramzid, Hossain Mirhendia, Mahmood Mahmoodia, Elaheh Shakibab.study on invasive fungal infections in immunocompromised patients. International J Infec Dis 2009;13: 97-102.

2 Pagano L, Caira M, Valentini CG, Posteraro B, Fianchi L Current therapeutic approaches to fungal infections in immunocompromised hematological patients. Southern Medical Journal 2010;103:245-7.

3 Paul L. Fidel Jr.,[1,*] Jose A. Vazquez,[2] and Jack D. Sobel[2] Candida glabrata: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to C. albicans. Clinical Microbiology Reviews 1999;12:80-96.

4 Bodey G.P, Mardani M,Hanna H.A,Boktour M, Abbas J, Girgawey E, Hachmem R.Y, Kontoyoiannis D.P, Raad. The epidemiology of Candida glabrata and Candida albicans fungaemia in immunocompromised patients with cancer Am.J Med. 2002;112:380-5.

5 Choi HK,Jeong SJ,Lee HS,Chin BS,Choi SH,Han SH,Kim MS,Kim CO,Choi JY, Song YG, Kim JM.Blood stream infections by Candida glabrata and Candida krusei:a single centre experience.Korean J Internal med 2009;24: 263-9.

6 Meis J,Petrou M,Bille T,Ellis D and Gibbs D.The Global Antifungal surveillance group 2000.A global evaluation of susceptibility of Candida species to fluconazole by disc diffusion. Diag Microbiol Infect Dis 36:215-233

7 Krcmery V,Barnes AJ.Non-albicans Candida species causing fungaemia:pathogenecity and

antifunagal resistance. J Hosp infect 2002; 50:243-60.

8. Hachem R,Hanna H,Kontoviannis D,Jiang Y,Raad I The changing epidemiology of invasive candidiasis: Candida glabrata and Candida krusei as the leading causes of candidemia in hematologic malignancy.Cancer 2008; 112:2493-9 .

9. Sheng-Yuan Ruana, Yu-Tsung Huangb, Chen-Chen Chuc, Chong-Jen Yud, Po-Ren Hsueh Candida glabrata fungaemia in a tertiary centre in Taiwan: antifungal susceptibility and outcomes nternational Journal of Antimicrobial Agents 34 (2009) 236–9.

10. Pfaller, M. A., and D. J. Diekema. 2004. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. Clin. Microbiol. Infect. 10(Suppl. 1):11-23.

11. Trick, W. E., S. K. Fridkin, J. R. Edwards, R. A. Hajjeh, R. P. Gaynes, and the National Nosocomial Infections Surveillance System Hospitals. 2002. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. Clin. Infect. Dis. 35:627-30.

12. Pfaller, M. A., S. A. Messer, L. Boyken, S. Tendolkar, R. J. Hollis, and D. J. Diekema. 2004. Geographic variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. J. Clin. Microbiol. 42:3142-46.

13. Diagnosis of invasive fungal infections in immunocompromised children H. J. Dornbusch1, A. Groll2 and T. J. Walsh3. Clin Microbiol Infect 2010; 16: 1328–1334

14. Ingi Lee, MD, MSCE; Neil O. Fishman, MD; Theoklis E. Zaoutis, MD, MSCE; Knashawn H. Morales, ScD; Mark G. Weiner, MD; Marie Synnestvedt, PhD; Irving Nachamkin, DrPH, MPH; Ebbing Lautenbach, MD, MPH, MSCE. Risk Factors for Fluconazole-Resistant Candida glabrata Bloodstream Infections *Arch Intern Med.* 2009;169:379-83.

15. Chris A.Gentry,Erin D,dayl j.Fluornoy Jamie S.Winner, Leonard N.Slater. A prospective observational study of risk factors for candida glabrata in an SICU. Role of Fluconazole. Hosp Pharmacy 2007;42:1139-44.

16. Trick WE, Fridkin SK, Edwards JR, et al. Secular trend of hospital acquired candidemia among intensive unit patients in the United States during 1989-1999. Clin Infect Dis 2002; 35:627-30.

17. Garbino J, Kolarova L, Rohner P, Lew D, Pichna P, Pittet D. Secular trends of candidemia over 12 years in adult patients at a tertiary care hospital. Medicine. 2002;81:425-33.

18. Pittet D, Wenzel RP. Nosocomial bloodstream infections: secular trends in rates, mortality, and contribution to total hospital deaths. Arch Intern Med 1995;155:1177-84.

19. Safran DB, Dawson E. The effect of empiric and prophylactic treatment with fluconazole on yeast isolates in a surgical trauma intensive care unit. Arch Surg 1997;132:1184-9.

20. Swoboda SM, Merz WG, Lipsett PA. Candidemia: the impact of antifungal prophylaxis in a surgical intensive care unit. Surg Infect 2003;4:345-54.

21. Blumberg H, Jarvis W, Soucie M, et al. Risk factors for Candidal bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multicenter study. Clin Infect Dis 2001;33:177-86.

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