Indian J Gastroenterol (March–April 2014) 33(2):165–168
DOI 10.1007/s12664-013-0439-6

SHORT REPORT

Is empiric therapy with fluconazole appropriate for esophageal candidiasis?

Kattiparambil Gangadharan Sajith · Amit Kumar Dutta · Rani Diana Sahni · Saritha Esakimuthu · Ashok Chacko

Received: 11 July 2013 / Accepted: 14 December 2013 / Published online: 16 January 2014

Abstract We studied the prevalence of fluconazole resistance in esophageal candidiasis. Patients with suspected esophageal candidiasis during gastroscopy underwent culture of white plaques. Minimum inhibitory concentration (MIC) >64 μg/mL of fluconazole for Candida was indicative of resistance. Sensitivity of itraconazole was tested in a subset of resistant strains. Sixty-five patients were included. Mean (SD) age was 50.03 (13.5) years and 67.7 % were males. Predisposing factors for candidiasis were found in 42 (64.6 %) patients. *C. albicans* was identified in 64 (97.4 %) patients and *C. glabrata* in one patient. Fluconazole resistance was seen in 38 (59.4 %) patients with *C. albicans* and also in the one patient with *C. glabrata.* All the fluconazole resistant isolates of *C. albicans* had MIC >128 μg/mL suggesting very high resistance. Twelve patients with fluconazole resistance had itraconazole resistance as well. The study shows a high rate of fluconazole resistance in patients with esophageal candidiasis.

Keywords Fluconazole · Fungal drug resistance · Itraconazole · Moniliasis

Introduction

Esophageal candidiasis is seen in 1 % to 6 % of immunocompetent patients undergoing upper gastrointestinal (GI) endoscopy [1, 2]. The incidence rises to about 40 % in immunocompromised hosts [3]. *Candida albicans* is the most common species implicated [4, 5]. Other species like *Candida krusei*, *Candida tropicalis*, and *Candida glabrata* may also cause esophageal candidiasis [4, 5]. Clinically, the infection may be asymptomatic or may cause odynophagia/dysphagia [6]. The infection may disseminate and result in serious illness in patients with impaired immunity [6]. Infectious Diseases Society of America 2009 Guidelines (IDSA) suggests fluconazole as the first line agent for the treatment of esophageal candidiasis [7]. Recent reports suggest that there is an increasing resistance of *C. albicans* to fluconazole [8]. Further, the frequency of non-albicans species like *C. krusei* which have high resistance to fluconazole is on the rise [9]. The aim of this study therefore was to assess the frequency of fluconazole resistance in patients with esophageal candidiasis.

Methods

Patients referred for upper GI endoscopy with symptoms related to upper GI tract to the Department of Gastroenterology, Christian Medical College, Vellore from January 2010 to January 2012 were included for the study. Patients with adherent white plaques in the esophagus on endoscopy were eligible to participate in this study. Those with history of candidiasis, prior treatment with fluconazole or recent use of broad spectrum antibiotics, were excluded. Biopsy/brushings for culture were obtained from all patients with endoscopic evidence of candidiasis. The diagnosis of esophageal candidiasis was confirmed when the culture of white plaques grew the fungus. Patient's demographic and clinical details were recorded on a pre-designed standard pro-forma. Blood investigations to assess the general condition of the patient (hemoglobin, albumin, blood sugars), and screening for HIV infec-

K. G. Sajith (✉) · A. K. Dutta · A. Chacko
Department of Gastrointestinal Sciences,
Christian Medical College, Vellore 632 004, India
e-mail: drsaji@yahoo.com

R. D. Sahni · S. Esakimuthu
Department of Microbiology, Christian Medical College,
Vellore 632 004, India

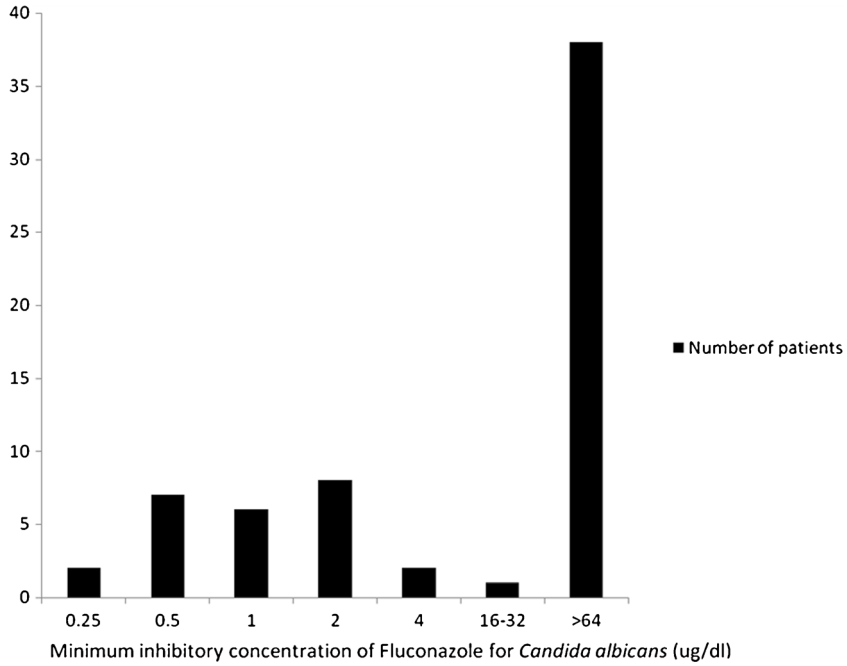

Fig. 1 Minimum inhibitory concentration of fluconazole for *Candida albicans*

tion, were also performed and recorded. Data regarding the use of immunosuppressants were documented. The study was approved by the institutional review board and ethics committee of our institute. Informed written consent was taken from all the study participants. Assuming a prevalence of fluconazole resistance in esophageal candidiasis to be 10 %, a sample size of 61 was required with a precision of 7.5 % and 95 % confidence interval. Continuous data is presented as mean with standard deviation. Categorical data is presented as percentages. Categorical data between two groups were compared using chi-square test. A p-value of ≤0.05 was considered significant. Statistical calculations were done using SPSS version 16 for windows.

Technique of fungal culture and antibiotic susceptibility

Esophageal biopsy samples with a clinical suspicion of candidiasis were ground aseptically and a gram stain and wet mount with calcofluor white were prepared and viewed using a fluorescent microscope at 365 nm wave length. Fungal cells fluoresce and are apple green in color. Specimens were then cultured on both Sabouraud's dextrose agar and Sabouraud's dextrose agar with gentamicin and chloramphenicol held up to 14 days [10]. Yeast-like colonies were confirmed with a Gram stain. The species identification of yeasts was done by performing a germ tube test and plating the isolates on a chromogenic media, CHROME agar Candida, Becton Dickenson Diagnostics (Catalog number 212961, Becton Dickenson, Maryland, MD, USA) [11]. All biopsy samples which grew *Candida* species were tested for fluconazole susceptibility. The minimum inhibitory concentration (MIC) of fluconazole on all isolates was performed according to the 2008 guidelines of Clinical and Laboratory Standards Institute (CLSI) (Wayne, PA, USA) using the microbroth dilution method (Approved standard—third edition. CLSI document M27-A3) [12]. Roswell Park Memorial Institute (RPMI)-1640 media obtained from Sigma Aldrich, India with a pH of 7.0±0.1 was used as basal media for the antifungal testing. Dilutions of fluconazole from 0.25 to 128 μg/mL were prepared in microtiter plates. The azole compounds used in the study namely fluconazole and itraconazole were obtained from Molekula Ltd. Technology House, UK (Molecula, Dorset, UK). ATCC 22019 *Candida parapsilosis* and ATCC 6258 *C. krusei* were used as test controls. An 80 % inhibition of growth was recorded as the MIC at 48 h. An observed MIC of ≤8 μg/mL was interpreted as susceptible to fluconazole, 16–32 μg/mL was interpreted as susceptible dose dependent, and ≥64 μg/mL was interpreted as resistant to fluconazole [12]. Twelve consecutive fluconazole resistant strains were chosen and tested against itraconazole. Dilutions of 0.06 to

Table 1 Factors associated with *Candida* infection and fluconazole resistance in this series of patients

Factors	*n*	Fluconazole resistance
Diabetes mellitus	17	13
Immunosuppressive therapy	11	6
Neoplasia	10	5
Chronic liver disease	8	3
Chronic renal failure	4	2
Hypothyroidism	4	4
HIV	4	3
Motility disorders	2	2

32 μg/mL of itraconazole were used to determine the MIC [12]. Isolates demonstrating an MIC of ≤0.125 μg/mL were interpreted as susceptible, 0.25–05 μg/mL was interpreted as susceptible dose dependent, and ≥1.0 μg/mL were interpreted as resistant [12].

Results

Sixty-seven patients had adherent white plaques suggestive of esophageal candidiasis on upper GI endoscopy and sixty-five were confirmed to have esophageal candidiasis by culture of white plaques. The mean (SD) age of these 65 patients was 50.03 (13.5) years and 67.7 % were males. Four patients had odynophagia and dysphagia while the rest had dyspeptic or reflux symptoms. Species identification showed that the fungal infection was caused by *C. albicans* in 64 patients and by *C. glabrata* in one patient. Resistance to fluconazole was seen in 38 (59.4 %) of the 64 patients with *C. albicans.* All the fluconazole resistant isolates of *C. albicans* had an MIC of >128 μg/mL suggesting significant resistance. Figure 1 shows the distribution of MIC of fluconazole for *C. albicans.* The single patient with *C. glabrata* was also resistant to fluconazole (MIC=64 μg/mL). Overall 39 (60 %) patients had esophageal candidiasis caused by a resistant strain. The predisposing factors for candidiasis are shown in Table 1. Of the 42 patients with predisposing factors, 14 had more than one factor. Diabetes mellitus was the commonest risk factor (40.5 %) followed by use of immunosuppressant drugs (26.2 %). The proportion of patients with fluconazole resistance was similar in patients with (27 of 42, 64.3 %) and without (11 of 22, 50 %) predisposing factors (p=0.27). In addition, the presence of an increasing number of predisposing factors was not significantly associated with resistance (p=0.7). Since majority of the *C. albicans* showed resistance to fluconazole, the second line sensitivity was done for itraconazole. Twelve consecutive fluconazole resistant strains were tested against itraconazole. All the fluconazole resistant isolates were resistant to itraconazole as well and showed a very high MIC of >128 μg/mL.

Discussion

Studies from India and other countries have reported a 5 % to 15 % prevalence of fluconazole resistance in patients with esophageal candidiasis [8, 13]. However, our study suggests that there is an alarming rise (60 %) in the frequency of fluconazole resistance. This raises serious concerns on the use of empiric fluconazole therapy especially so in immunocompromised patients who are at risk of developing fungemia. The resistance could be related to fungal factors (resistance causing mutations or species with inherent fluconazole resistance), drug factors (low dose, prior therapy, drug interactions) and/or, patient related factors (compliance, immune status) [14]. Itraconazole is recommended as a second line drug for mucosal candidiasis in the IDSA guidelines [7, 15]. However, in our study, all 12 fluconazole resistant isolates tested were also resistant to itraconazole. Voriconazole is recommended for *C. krusei* and *C. glabrata* [7]. The sensitivity of newer azoles like voriconazole in patients with fluconazole resistance was not evaluated in the present study. As cross resistance has been demonstrated among azoles, resistance to these drugs is also a possibility [16]. Another interesting observation was the predominance of *C. albicans* species in our patients. This is in contrast to other Indian studies that have shown *C. tropicalis* accounting for 10 % to 40 % of cases of esophageal candidiasis and candidemia [1, 13]. It could be related to the selection of resistant species in the background of increased usage of antifungals. Reports suggest that there are variations in the frequency of different *Candida* species causing infection in different geographical regions and their drug sensitivity patterns also vary. In their systematic review of the distribution of species causing candidemia, Falagas et al. reported a significant geographic variation and stressed on the need of local epidemiological data [17]. A recent study from Pakistan showed that *C. tropicalis* was the commonest species in adults while *C. albicans* was predominantly seen in children. Majority (98 %) of their patients with *Candida* had a low MIC of fluconazole supporting empiric therapy with fluconazole for invasive candidiasis in that country [18]. Based on the results from our study, we suggest a revision in the treatment policy in patients with esophageal candidiasis in those areas where fluconazole resistance is prevalent. Considering that more than half of the study population were resistant to fluconazole and the fact that patients with esophageal candidiasis are seldom followed up, it would be ideal to collect the specimen for culture and antifungal sensitivity as suspected and when it is suspected during endoscopy. If this option cannot be considered, a repeat endoscopy may be considered to confirm the clearance of infection (at least in immunocompromised population). An echinocandin or amphotericin B is recommended by IDSA guidelines as the alternate option for esophageal candidiasis [7]. A major limitation of our study is that the sensitivity of newer azoles, echinocandins, or amphotericin was not tested. This would have helped us to determine the optimal antifungal agent for empiric therapy. The correlation between in vitro and in vivo studies also needs to be addressed in further studies. In conclusion, the frequency of resistance of *C. albicans* to fluconazole is high among Indian patients. It may be prudent to assess the sensitivity pattern of fluconazole in different geographical regions before formulating guidelines for empirical therapy.

Acknowledgments We would like to acknowledge the Retired Professor, Department of Microbiology, Dr Mary Mathews, Professor and Former Head, Department of Microbiology, Christian Medical College, Vellore and Dr V Balaji. Professor and Head, Department of Microbiology, Christian Medical College, Vellore, for providing the laboratory facility for the study.

The study was funded by the Research Grant from Christian Medical College, Vellore Fluid Research Fund.

Conflict of interest statement K G Sajith, A K Dutta, R D Sahni, S Esakimuthu, and A Chacko have no conflicts of interest to declare.

Ethics statement The authors affirm that the study was performed in a manner conforming with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning Human and Animal Rights, and that the authors followed the policy concerning Informed Consent as shown in Springer.com.

References

1. Badarinarayanan G, Gowrisankar R, Muthulakshmi K. Esophageal candidiasis in non-immune suppressed patients in a semi-urban town, South India. Mycopathologia. 2000;149:1–4.
2. Yoshikawa T, Naito Y, Oyamada H, et al. Esophageal candidiasis. Gastroenterol J. 1988;23:363–70.
3. Porro GB, Parente F, Cernushi M. The diagnosis of esophageal candidiasis in acquired immune deficiency syndrome: is endoscopy always necessary? Am J Gastroenterol. 1989;84:143–6.
4. Mathieson R, Dutta SK. Candida esophagitis. Dig Dis Sci. 1983;28: 365–70.
5. Kodsi BE, Wickremesinghe PC, Kozinn PJ, Iswara K, Goldberg PK. Candida esophagitis: a prospective study of 27 cases. Gastroenterology. 1976;71:715–9.
6. Baehr PH, McDonald GB. Esophageal infections: risk factors, presentation, diagnosis and treatment. Gastroenterology. 1994;106:509–32.
7. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the infectious diseases society of America. Clin Infect Dis. 2009;48:503–35.
8. Wilheim AB, Miranda-Filho Dde B, Nogueira RA, Rêgo RS, Lima Kde M, Pereira LM. The resistance to fluconazole in patients with esophageal candidiasis. Arq Gastroenterol. 2009;46:32–7.
9. Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N Engl J Med. 1991;325:1274–7.
10. Sutton DA. Specimen collection, transport, and processing: mycology. *In*: Murray PR, (ed). Manual of Clinical Microbiology, Vol 2. 9th ed. Washington DC: ASM; 2007. p. 1728–36.
11. Iyampillai T, Michael JS, Mathai E, Mathews MS. Use of CHROMagar medium in the differentiation of *Candida* species: is it cost-effective in developing countries? Ann Trop Med Parasitol. 2004;98:279–82.
12. Espinel-Ingroff AV, Pfaller MA. Susceptibility testing methods; yeasts and filamentous fungi. *In*: Murray PR, (ed). Manual of Clinical Microbiology, Vol 2. 9th ed. Washington DC: ASM; 2007. p. 1972–86.
13. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary centre of North India: 5 year study. Infection. 2007;35:256–9.
14. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. Antimicrob Agents Chemother. 1995;39:1–8.
15. Eichel M, Just-Nubling G, Helm EB, Stille W. Itraconazole suspension in the treatment of HIV-infected patients with fluconazole-resistant oropharyngeal candidiasis and esophagitis. Mycoses. 1996;39:102–6.
16. Panackal AA, Gribskov JL, Staab JF, Kirby KA, Rinaldi M, Marr KA. Clinical significance of azole antifungal drug cross resistance in *Candida glabrata*. J Clin Microbiol. 2006;44:1740–3.
17. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various nonalbicans candida species among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis. 2010;14:954–66.
18. Farooqi JQ, Jabeen K, Saeed N, et al. Invasive candidiasis in Pakistan: clinical characteristics, species distribution and antifungal susceptibility. J Med Microbiol. 2013;62:259–68.