SIGNIFICANCE OF ISOLATION AND DRUG SUSCEPTIBILITY TESTING OF NON-*CANDIDA ALBICANS* SPECIES CAUSING OROPHARYNGEAL CANDIDIASIS IN HIV PATIENTS

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Abstract. Oropharyngeal candidiasis (OPC) continues to be a common opportunistic infection in patients infected with Human Immunodeficiency Virus (HIV) and is predictive of increasing immunosuppression. Though *Candida albicans* remains the predominant isolate, a rise in the frequency of isolation of non-*albicans Candida* (NAC) species is being observed. The levels of virulence and the sensitivities to available antifungal drugs vary among these species. Of 340 HIV seropositive patients in this study, 132 (38.8%) had oral lesions suggestive of candidiasis. Samples were collected from the lesion using sterile cotton swabs. Isolation and speciation were done by standard techniques. Antifungal drug susceptibility testing was done by macro broth dilution. The total number of *Candida* isolates was 135, of which, 45 (33.3%) were NAC species and 90 were *C.albicans* (66.6%). Of the NAC species, *C. dubliniensis* was the predominant pathogen (22,48.9%). Antifungal susceptibility testing showed that 14 (31.1%) of the NAC species and 11 (12.2%) of *C. albicans* were resistant to fluconazole (MIC > 8 µg/ml). A very high MIC of >32 µg/ml was noted among the NAC species resistant to fluconazole.

INTRODUCTION

The continuing acquired immunodeficiency syndrome (AIDS) epidemic, malignancies and aggressive chemotherapeutic interventions have created an extremely vulnerable population of immunocompromised patients who are highly susceptible to a variety of microbial infections including fungal infections (Jabra-Rizk *et al,* 2000).

Oropharyngeal candidiasis (OPC) is the most common fungal infection in HIV infected patients and has been identified as a clinical predictor for progression to AIDS (Vargas and Joly, 2002). Though *Candida* majority of candida infections are caused by *Candida albicans*, reports of non-*albicans Candida* (NAC) infections have begun to appear (Vargas and Joly, 2002). The increasing use of fluconazole to treat HIV patients with OPC has resulted in a change in the prevalence of different candida species and the emergence of azole resistance (Powderly *et al*, 1999). Long term use of azoles may lead to selection of less sensitive species, such as *C. krusei* and *C. glabrata,* and the development of resistance in previously susceptible *Candida* strains (Hajjah *et al*, 2004).

The advent of effective anti-retroviral therapy for the treatment of HIV has led to a scenario in which antifungal strategies are likely to become more effective (Powderly *et al*, 1999). However, the increase in frequency

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of NAC infections coupled with high levels of resistance to common antifungal drugs are a cause for concern in the prophylaxis and treatment of OPC in HIV patients.

MATERIALS AND METHODS

The present study was carried out at Karnataka Institute of Medical Sciences (KIMS) Hospital, Hubli, Karnataka, India. A total of 340 HIV seropositive patients admitted to the medical wards were included in the study. After obtaining informed consent, two samples were collected from the oral lesion with sterile cotton swabs. One was used for Gram's stain and other was inoculated on Sabourauds dextrose agar (SDA) slant. The slants were incubated at 37ºC and observed for the appearance of colonies suggestive of candida. These colonies were further processed and identified to species level as per standard methods (Rippon, 1988).

By using germ tube formation was carried out along with colony morphology on CHROM agar (Hi Media, Mumbai) (Sullivan and Coleman, 1998), differential growth at 37ºC and 42ºC, corn meal agar morphology and sugar assimilation profile.

After speciation, antifungal drug susceptibility testing against ketoconazole and fluconazole was performed by macro broth dilution technique according to NCCLS guidelines (Corimican and Pfaller, 1996). The MIC was calculated for each isolate based on which the isolates were classified as sensitive, intermediate or resistant to ketoconazole and fluconazole.

RESULTS

Of the 340 HIV seropositive patients studied 132 (38.8%) had oral lesions suggestive of OPC. The Gram's stain was positive for yeast in 70 (53%) cases. Candida was isolated from SDA culture in all 132 (100%) samples. Of these, 3 revealed a mixture of 2 species, which were identified using CHROM agar, so the total number of isolates was 135. Of these 135 isolates, non-*albicans Candida* (NAC) species were found in 45 isolates (33.3%), comprised of *C. dublinienses* 22 (48.9%), *C. krusei* 9 (20%), *C. parapsilosis* 5 (11%), *C. stellatoidae* 3 (6.7%), *C. tropicalis* 4 (8.9%) and *C. guillerimondi* 2 (4.9%) isolates. The remaining 90 isolates (66.6%) were *C. albicans*.

Antifungal susceptibility testing (Table 1) showed 14 (31.1%) NAC isolates and 11 (12.2%) *C. albicans* isolates were resistant to fluconazole. A high MIC (>32 µg/ml) was found in 7 NAC isolates (15.5%), of which 3 (6.61%) were *C. dubliniensis*, 2 (4.4%) each were *C. krusei* and *C. parapsilosis.* Such a high MIC was seen in only 3 (3.3%) *C. albicans* isolates. Resistance to azoles was more common among NAC isolates than *C.albicans* isolates. Against ketoconazole 12 (26.6%) NAC isolates and 9 (10%) *C. albicans* isolates showed resistance. Of these 12 (26.6%) resistant NAC isolates, 4 (8.8%) were *C. dubliniensis*, 3 (6.6%) were *C. krusei* and 2 (4.4%) each were *C. parapsilosis, C. tropicalis* and 1 (2.2%) was *C. stellatoidea.*

DISCUSSION

The incidence of OPC among HIV patients in our study was 38.8%. One hundred ten patients (83.3%) had pseudomembrane type lesions. Twenty-two (16.6%) had erythematous lesions. The high frequency of candidiasis in immunocompromised individuals emphasizes that a fully functional immune system is needed to prevent candida colonization (Cannon *et al*, 1995).

The predominant species isolated was *C. albicans* (90,66.6%). A large number of NAC isolates (45, 33.3%) was observed in our study. KIMS Hospital is a tertiary care hospital and referral center located in northern

Table 1
MIC of isolates to fluconazole and ketoconazole.

Species	MIC (µg/ml)								Total
	Sensitive			Intermediate		Resistant			
	Flu[a]	Ket[b]		Flu[a]	Ket[b]	Flu[a]		Ket[b]	
	0.125-0.5	0.03-0.125	0.25-0.5	1-8	1-4	16-32	>32	> 4	
C. albicans	50	30	32	29	19	8	3	9	90
C. dubliniensis	12	7	5	5	6	2	3	4	22
C. krusei	3	2	2	2	2	2	2	3	9
C. parapsilosis	2	2	1	0	0	1	2	2	5
C. tropicalis	2	0	2	1	0	1	0	2	4
C. stellatoidea	1	1	1	1	0	1	0	1	3
C. guillerimondi	1	1	0	1	1	0	0	0	2
Total	71	43	43	39	28	15	10	21	135

[a]Flu-fluconazole; [b]Ket-ketoconazole

Karnataka, providing anti-retroviral therapy (ART) and treatment for opportunistic infections in AIDS patients. Most of our inpatients are referred from peripheral centers; they are either terminally ill or are patients not responding to therapy. An increase in the number of NAC isolates may be attributed to these factors, indicating prior use of fluconazole in these patients. Prolonged use of fluconazole may be responsible for the development of highly resistant microorganisms and may impact the ecology of oral yeast species by effecting a shift toward species with innate or acquired azole resistance (Martinez *et al*, 2002).

There is a tendency to report all germ tube positive isolates as *C. albicans*, but *C. dubliniensis* and *C. stellatoidea* are also germ tube positive, which unless speciated properly may lead to an error in reporting. Among germ tube negative *Candida*, *C. krusei* and *C. glabarata* are known to be resistant to azoles (Hajjah *et al*, 2004) and hence need proper identification. A mixture of two species was noted in three of our patients, which were identified by CHROM agar inoculation.

High level resistance to azoles among NAC isolates emphasizes the need for species directed treatment. Topical therapy should be considered during early OPC and systemic azole therapy should be reserved for more severe cases with esophageal involvement. Prolonged fluconazole treatment influences the oral microbial ecology in these patients. These isolates may show decreased susceptibility to fluconazole but may still be susceptible to newer azoles, sush as voriconazole and itraconazole (Kirkpatrick *et al*, 1998). As the immunocompromised population continues to grow in number, proper medical therapy becomes paramount (Jabra-Rizk *et al*, 2000).

The evolving importance of NAC species in HIV requires incorporation of standard techniques for speciation of *Candida* as a routine procedure. Simple additional techniques, such as CHROM agar inoculation and growth at differential temperatures can be helpful in HIV patients with low CD4 counts for presumptive identification of clinically important *Candida* species (Powderly *et al*, 1999).

The indentification of species is necessary not only for therapeutic purposes, but also as a means to determine the incidence

and prevalence of these species, as well as to determine their role in infection, especially in invasive and systemic infections.

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