Original Article

Study of Candidiasis in HIV seropositive patients

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Abstract

Candida species are the most common cause of fungal infections. Candida species are opportunistic yeast like fungi. They are ubiquitous and have potential to cause human disease under specific circumstances and conditions. **Materials and methods**: A total of 172 Candida species were isolated from various clinical samples like oral swab, vaginal swab, urine, sputum, bronchial aspirate and other samples. All these samples were collected from patients having signs and symptoms of candidiasis. All the isolates were identified by using urease test, germ tube test, Dalmau plate culture method, growth on hichrome Candida agar and Growth at 45°C. Antifungal susceptibility test was done on all the isolates by disk diffusion method (NCCLS M44-A). **Results:** Out of 172 total isolates, 112 were identified as Candida albicans, 41 as Candida tropicalis, 05 as Candida krusei, 04 as Candida dubliniensis and 10 as Candida glabrata.
Keywords: Candida, Dalmau plate culture method, HIV, Antifungal susceptibility testing

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INTRODUCTION

Fungal infections are known to affect the immunocompromised hosts. Modernization of medicine and progressive HIV pandemic has provided ample ground for the fungal pathogens.[1] The advent of HIV and pandemic of AIDS have greatly increased the number of immunocompromised individuals susceptible to a wide variety of pathogens including mycoses due to yeast species of genus Candida.[2] Candida is the most common fungal agent encountered in human disease. Candidiasis is often among the earliest detectable clinical manifestations of HIV seropositivity. Oral candidiasis occurs in about 95% of HIV seropositive individuals at some stage of their illness. It is highly predictive of progression to AIDS.[2]

The common species found to cause human infections are C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. lusitaniae, C. kefyr, C. gullermondii and C. dubliniensis. Candida albicans is the most frequently reported causative agent associated with various lesions in immunocompromised individuals. The frequency of infections caused by non albicans Candida (NAC) is also increasing. The increased frequency of these non albicans species is probably secondary to an alteration in the flora induced by the use of systemic azoles[2]. These species are also shown to have decreased susceptibility to antifungal agents. C. krusei and C. glabrata have innate resistance to fluconazole[3,4,27,28]. Presence of candidiasis may serve as clinical marker for underlying immunosuppression. Hence, identification of Candida species is very important in the laboratory. Identification of species has therapeutic significance, allowing use of appropriate antifungal agents and preventing emergence of drug resistance.

The disk diffusion method of antifungal susceptibility testing is relatively simple, reliable, reproducible method. Resistance to most of the common antifungal drugs is emerging, so rapid in vitro susceptibility test is required to guide the selection of antifungal therapy for successful antifungal susceptibility test was done on all the isolates by disk diffusion method (NCCLS document M44-A). This study was done to isolate various species of Candida from various clinical

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samples, identify them by using various methods and to perform the antifungal susceptibility test of all the isolates.

MATERIALS AND METHODS

A total of 172 Candida strains were isolated from various clinical specimens like oral swab, vaginal swab, urine (midstream), sputum, tracheal aspirate and pus. Samples were collected from HIV seropositive patients who were having clinically evident lesions suggestive of candidiasis. For oral and vaginal sample collection, two swabs were collected, one for Gram staining and one for culture. For oral swab, vaginal swab, sputum, tracheal aspirate and pus primary smears were prepared and Gram staining was done and findings of Gram staining were noted. For urine sample midstream urine sample was collected and wet mount of urine was examined for the presence of yeast cells and pseudohyphae if present. Sabouraud dextrose agar (SDA) (Himedia, Mumbai, India) slant was used as culture medium. Inoculation of SDA slants was done by using various clinical samples.

The slants were observed daily for presence of growth for seven days. When growth was observed on SDA slants, colony smear was made and stained with Gram stain method. Further tests done for the identification were urease test, germ tube test, Dalmau plate culture method, identification by growth on chrome agar and growth at 45ºC. Antibiotic susceptibility test was done on all the isolates.

Germ tube test[5]: A small portion of isolated colony suspended in test tube containing 0.5 ml of human serum. The test tube was incubated at 35ºC for not more than two hours. A drop of yeast-serum suspension was taken on microscope slide overlaid with coverslip and examined under 45X objective lens for presence or absence of germ tube.

Dalmau plate culture method[6]: A heavy inoculum of yeast was streaked across plate containing cornmeal agar with tween 80 and coverslip was placed over it. The streak should project beyond coverslip. Plates were incubated at 25ºC for 24 to 48 hours. Examination was done for presence of chlamydospores, arrangement of pseudohyphae under low and high power.

Use of Hichrome Candida agar[7,8,9]: Streaking was done on Hichrome Candida agar (Himedia, Mumbai, India) with a isolated colony of yeast. Colour and morphology of the colony was observed after 48 hours of incubation.

Urease test[10]: Christensen's Urease agar (Himedia, Mumbai, India) was inoculated with isolated colony of yeast. Test tubes were incubated at 30 ºC for one week. Change of colour to pink is considered as a positive test.

Growth at 45 ºC[11]: All germ tube test positive isolates were inoculates on SDA plate. The plates were incubated at 45 ºC for a week. If abundant growth was present, the isolate was considered as C. albicans. If there is no growth, the isolate was considered as C. dubliniensis.

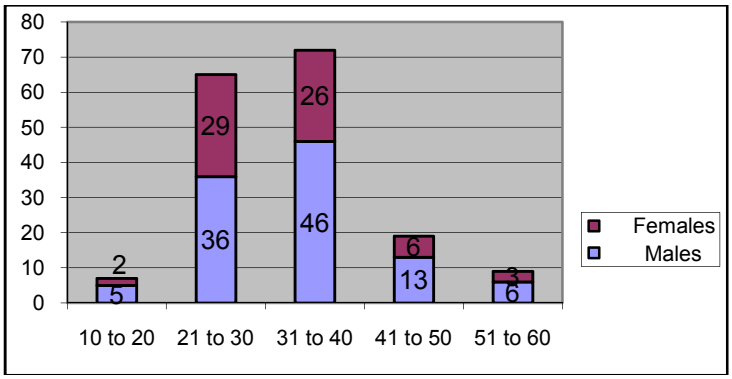
Antifungal susceptibility test[12,13]: The susceptibility of the Candida species to fluconazole was done by NCCLS M44 A disk diffusion method. 25 µg disk of fluconazole (Himedia, Mumbai, India) was used to perform antifungal susceptibility test. Isolated colony was suspended in sterile saline and the turbidity of inoculums was adjusted to match 0.5 Mcfarland density standard. With the help of sterile cotton swab moistened with the inoculum suspension lawn culture was done on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue. Fluconazole disc was applied on the surface of the medium. Plates were incubated at 37 ºC for 24 hours and diameter of zone of inhibition was noted.

RESULTS

In this study majority of patients were male 62% and females were 38%. Maximum patients (72) belong to age group 31-40 years (male 46 and female 26) followed by age group 21-30 years, which had 65 patients (36 male and 29 female).

Table 1

Age group	No of cases	Males	Females
10-20	07	05	02
21-30	65	36	29
31-40	72	46	26
41-50	19	13	06
51-60	09	06	03
Total	**172**	**106**	**66**



Urease test: All the isolates were urease negative.

Germ tube test: The test was given positive by 116 isolates from which 112 were Candida albicans and 04 were further identified as Candida dubliniensis on the basis of growth at 45°C, Dalmau plate culture method and morphology on Chrome agar Candida.

Dalmau plate culture method: In this study, 112 were Candida albicans, 41 were Candida tropicalis, 05 were Candida krusei, 04 were Candida dubliniensis and 10 were Candida glabrata.

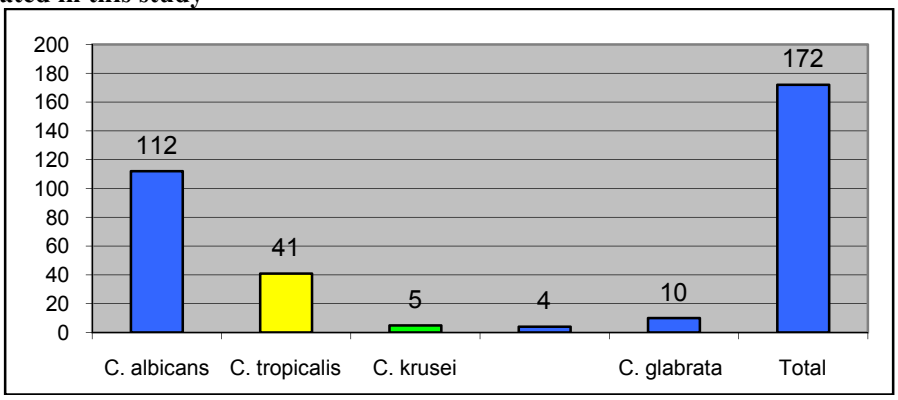
Table 2: Morphology of various Candida species on Dalmau plate culture method

Species	Number	Observation
C. albicans	112	Elongated pseudohyphae with large clusters of blastoconidia at junctures between cells with terminal chlamydospores.
C. tropicalis	41	Abundant pseudohyphae and abundant branching, often radiating with clusters of blastoconidia at the center.
C. krusei	05	"Crossed- matchsticks" appearance
C. dubliniensis	04	Abundant chlamydospores, abundant pseudohyphae, some true hyphae, clusters of blastospores along pseudohyphae
C. glabrata	10	No pseudohyphae

Results of morphology on chrome agar Candida

Species	Morphology on chrome agar
C. albicans	Light green colonies.
Candida tropicalis	blue coloured colonies
Candida dubliniensis	Dark green colonies
Candida krusei	Purple colonies
Candida glabrata	Cream to white coloured colonies.

Various species isolated in this study



Results of Growth at 45ºC: This test was done on all the isolates which showed germ tube test positive

Total germ tube positive isolates	Presence of growth at 45°C and 37°C	Presence of growth at 37°C only
116	112	04

Those isolated which show growth at 37°C only are identified as C. dubliniensis. Results obtained by all these methods of identification match with each other.

Results of antifungal susceptibility testing: Resistance pattern of various Candida species against fluconazole

Candida species	Fluconazole	Total
C. albicans	41(36.6%)	112
C. tropicalis	16(39%)	41
C. glabrata	03(30%)	10
C. krusei	05(100%)*	05
C. dubliniensis	1(25%)	04
Total	**66(38.3%)**	**172**

*Candida krusei have innate resisiance to fluconazole

Out of 112 C. albicans isolates, 41(36.6%) were resistant to fluconazole, out of 41 C. tropicalis isolates, 16(39%) were resistant, out of 10 C. glabrata isolates, 03(30%) were resistant to fluconazole, 05 out of 05 C. krusei isolates were resistant to fluconazole and 01 out of 04 C. dubliniensis was resistant to fluconazole. Overall, 66(38.3%) Candida iaolates were resistant to fluconazole.

DISCUSSION

Though Candida albicans is responsible for most of the cases of candidiasis, there has been striking increase in the non albicans Candida species primarily C. tropicalis, C. krusei, C. parapsilosis and C. glabrata. The isolation and identification of yeast becomes more and more necessary for the choice of adequate therapy. This is particularly important in view of the development of resistance to azole group of antifungal agents in previously susceptible C. albicans strain and of the different antifungal susceptibility patterns of non albicans species. We undertook the present study to find out various Candida species causing various clinical conditions in HIV seropositive individuals and their susceptibility to commonly used azole i.e. fluconazole. The majority of patients presented with candidiasis belong to age group 21-40 years. The mean age group was 33.79 years. It is well documented that cases of AIDS belong to sexually active people. The mean age group was 27 in study of Barone *et al*[14], 30.5 in Schiodt *et al*[15], 38.5 in Sangeorzan *et al*[16]. In Gram staining of the primary smears, presence of pseudohyphae indicates the tissue invasion by Candida species and is of more significance. Germ tube test was positive for 116 isolates. Out of these 116 isolates, 112 were C. albicans and 04 were C. dubliniensis. C. albicans and C. dubliniensis can be differentiated by Growth at 45ºC, Growth on chrome agar and by Dalmau plate culture method. Identification by Dalmau plate method was done as per the guidelines given in VII th National Workshop on simple diagnostic methods in clinical microbiology[6]. Recognition of yeast morphologies on Dalmau plates requires some experience but once this is mastered the method is simple and reliable. The findings of identification by morphology on chrome agar Candida matches with various studies done by Sanjeev kumar *et al*[17], Sayyada ghufrana nadeem *et al*[18] and Odds and Bernaerts[19]. This method is simple, rapid, cost effective method of identification of various Candida species.

Percentage of Candida albicans in various studies

Study	Percentage of Candida albicans
Usha arora *et al*[20]	62.5%
V P Baradkar and S Kumar[21]	70%
Franker *et al*[22]	84%
Barone *et al*[14]	87%
Walmsley *et al*[23]	79.4%
Our study	**65.1%**

Fluconazole resistance pattern of Candida species in various studies

Our study	Mondal s *et al*[24]	Abhijit tiwari *et al*[25]	Zarei Mahmoudabadi A *et al*[26]
38.3%	28.3%	37.6%	48.4%

In our study we found all candida krusei strains are resistant to fluconazole. This is because C. krusei have innate resistance to fluconazole. Revankar *et al*[27], Narain *et al*[28] also have similar finding in their studies.

CONCLUSION

In our study, 172 HIV seropositive cases having clinical features of Candidiasis were included. Presence of candidiasis indicates underlying immunosuppression in the patient. Candida albicans was the major (65.1%) Candida species isolated. Correlation between Germ tube test and C. albicans speciation was good. Non albicans Candida accounted for 34.9 % of total Candida isolates. Recognition of yeast morphologies on Dalmau plates requires some experience but once this is mastered the method is simple and reliable. Identification of various Candida species by morphology on CHROMagar is a rapid, reliable, inexpensive method. 38.3% of the all the Candida species were resistant to fluconazole. Candida krusei has innate resistance to fluconazole. NCCLS M44A disk diffusion method is easy, reliable method to perform antifungal susceptibility testing. To conclude Candida albicans was the major species out of all Candida isolates in our study however the emergence of non albicans Candida species cannot be overlooked. There is emergence of fluconazole resistance and C. krusei having

innate resistance to fluconazole, speciation of Candida isolates and assessment of its susceptibility to antifungal agents is necessary in every case.

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