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RESEARCH ARTICLE

CHARACTERISATION OF CANDIDA SPECIES FROM ORAL THRUSH IN HIV SEROPOSITIVE CASES

¹Dr. Spriha Smriti, ²Dr. Pratulaya Nandan and ³Dr. Ranjan Kumar Srivastava

¹Tutor, Deptt., of Microbiology, P.M.CH, Patna

²Assistant Professor, Deptt of Microbiology, P.M.C.H, Patna

³Associate Professor, HOD, Deptt., of Microbiology, P.M.C.H, Patna

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ABSTRACT

Introduction: Globally 36.7 million people are infected with AIDS. India alone has 2.1 million patients with HIV infection. Oral Candidiasis is the most common opportunistic fungal infection. In HIV infection, the isolation of non-*albicans* from oral Candidiasis is on a rise. Hence it is necessary to identify *Candida albicans* as well as non-*albicans* species too. **Method:** The present study was undertaken on 100 patients to identify the isolates and correlate them to CD4+ lymphocyte count for the period of one year. KOH mount and Gram's stain done for demonstration of yeast like cells. Isolation was done on SDA media. Isolation was further processed by germ tube test and confirmed by fermentation and sugar assimilation test. Culture was grown on Chrom agar and CD4+ T cell count done. **Result:** Specificity of Gram's stain and KOH mount was 76% and 64% respectively. SDA yielded 100% growth. Germ tube was positive in all *C. albicans* and *C.dublinensis* cases. *C.albicans* was the most common species isolated (28.3%), followed by *C.tropicalis* (26.66%), *C.guilliermondii* (17.69%), *C.dublinensis* (10.61%), *C.krusi* (7.07%), *C.parapsilosis* (6.19%), *C.kefyr* (3.53%), least isolated species *C.glabrata* (0.88%). Mean CD4+ T lymphocyte count was 125.28±78.45 cells/μl of blood. **Discussion:** In 2007 work in Maulana Azad Medical College, New Delhi found that *C. albicans* was most predominant 59.3%, followed by *C.glabrata*(14.8%), *C.parapsilosis* (11.8%), *C.guilliermondii* and *C.tropicalis* 1% each. In 2009 Kumar G.Menon CP, Thangam et al showed that out of 54 cases of HIV positive patients, 44% were infected with *C.albicans* and 56% were non-*candida albicans*. **Conclusion:** There is an increase in occurrence of non-*candida albicans* species in Oral Candidiasis. Oral Candidiasis affects patient's compliance of ART and nutritional intake.

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INTRODUCTION

Globally 36.7 million people are infected with AIDS. India alone has 2.1 million patients with HIV infection. Oral Candidiasis is the most common opportunistic fungal infection. In HIV infection, the isolation of non-*albicans* from oral Candidiasis is on a rise. Hence it is necessary to identify *Candida albicans* as well as non-*candida albicans* species. HIV is caused by human immunodeficiency virus 1 and 2. The virus targets the cells with CD4 surface molecules majority being T lymphocyte. Progressive decline in immunological responses makes them susceptible to opportunistic infection. *Candida* normal commensal in 20-50% of Indian population. In HIV infection colonisation increases upto 81% (Singh, 1999). With normal or nearly normal CD4 count vaginal candidiasis occurs, oral candidiasis occurs when CD4 count is less than 300 cells/mm², oesophageal candidiasis occurs when CD4 count is less than 100cells/mm².

*Corresponding author: Dr. Spriha Smriti,
Tutor, Deptt., of Microbiology, P.M.CH, Patna.

The present study was done to isolate *Candida* from oral candidiasis in HIV seropositive cases and speciate them by various tests and correlate them to CD4 count.

MATERIALS AND METHODS

100 HIV positive cases with clinically suspected oral candidiasis of all age group and both sexes attending ART Patna Medical College and Hospital after being tested reactive in VCTC centre of Patna Medical College an Hospital. Tests were done in the Microbiology department of Patna Medical College, Patna. Patient were informed and full consent was taken from them at the time of sample collection.

Collection of sample: Two sterile cotton tipped wooden swabs moistened with saline was used to swab and scrap the lesions from mouth. Swabs are kept in sterile container and immediately transported to microbiology laboratory. One swab was used for KOH mount and Gram stain. Second swab was inoculated on SDA (Sabouraud dextrose agar).

On KOH mount- Material is placed on clean glass slide, few drops of 10% KOH added on it and cover slip is placed. On observation under 10x and 40x we see for budding yeast cells and pseudohyphae. On Gram staining also we see under 100x for Gram positive budding yeast like fungus, pseudohyphae and inflammatory cells. On SDA Media- After inoculation on SDA and incubated at 25 °C and 37 °C and observed for growth for 24hrs to 72 hrs upto 3weeks to report for no growth. Further if growth occurs then from it Germ tube test (Reynold's Braude Phenomenon) – It helps in presumptive identification of *C.albicans* and *C. dubliniensis* if germ tube is positive within 2hrs of inoculation.

Inoculation on cornmeal agar: Inoculation is done by Dalmat technique. We observed for Chlamydo-spore. Further tests for species identification like carbohydrate fermentation and sugar assimilation test was done.

RESULTS

The below table that the study group consists of 100 HIV seropositive patients and 50% of patients are between age group 31-45yrs, 33% between 16-30yrs age group.

Table 1. Age wise distribution

Age group(years)	No of cases	Percentage
0-15	0	0
16-30	33	33
31-45	50	50
46-60	16	16
61-75	1	1
Total	100	100

Table 2. Sex wise distribution

Sex	No of cases	Percentage
Male	54	54
Female	46	46
Total	100	100

The above table showed males constituting 54% and females 46% of the study group. Male to female sex ratio 1.17:1

Table 3. Antiretroviral therapy in patients

ART mode	Number	Percentage
On ART	64	64
No ART	36	36
Total	100	100

The above table shows 64% of HIV seropositive patients were on antiretroviral therapy

Table 4. Occurrence of oral candidiasis

Occurrence of oral candidiasis	Number	Percentage
First episode	67	67
Recurrent episode	33	33
Total	100	100

Of 100 seropositive cases 67% had first episode of oral candidiasis and 33% had recurrent candidiasis.

Table 5. CD4 count distribution

CD4+count (cells/μl)	1 st episode		Recurrent episode	
	No	%	No	%
>200	8	11.94	2	6.06
150-250	13	19.40	7	21.21
<150	46	68.66	24	72.73
Total	67	100	33	100

Mean CD4 count is 125.28 cells/μl

Mean CD4 count is 125.28 cells/μl. First episode of oral candidiasis occurred in 46 patients with CD4 count <150cells/μl while patients with CD4 <150 cells/μl had recurrent oral candidiasis.

Table 6. Observation of Candida on KOH, Gram and Culture

Procedure	Number	Percentage
Gram stain	76	76
KOH mount	64	64
Culture on SDA	100	100

100% of samples yielded growth of candida on SDA. Budding yeast cells and pseudohyphae was demonstrated in 76% by Gram stain and 64% by KOH mount.

Table 7. Occurrence of different species of Candida in isolates

Species isolate	Number	Percentage
<i>C.albicans</i>	32	28.31
<i>C.dubliniensis</i>	12	10.61
<i>C.tropicalis</i>	29	25.66
<i>C.guilliermondii</i>	20	17.69
<i>C.parapsilosis</i>	7	6.19
<i>C.krusei</i>	8	7.07
<i>C.kefyr</i>	4	3.53
<i>C.glabrata</i>	1	0.88
Total	113	100

The most common species isolated was *C.albicans* 32(28.31%). Non *Candida albicans* isolates were 81(71.69%) of which the most common species was *C. tropicalis* and so on given in the table.

Table 8. Occurrence of different species in relation to CD4+T cells

Species isolated	CD4 + T cell count (cells/μl)		
	<150	150-250	>250
<i>C.albicans</i>	25	4	3
<i>C.dubliniensis</i>	9	3	0
<i>C.tropicalis</i>	19	7	3
<i>C.guilliermondii</i>	12	6	2
<i>C.parapsilosis</i>	6	0	1
<i>C.krusei</i>	5	2	1
<i>C.kefyr</i>	3	1	0
<i>C.glabrata</i>	1	0	0
Total	80	23	10

The common isolates at CD4 count >150 cells/μl were *C.albicans*, *C.tropicalis* and *C.guilliermondii*. CD4 count <150 cells/μl there is increased occurrence of other species such as *C.dubliniensis*, *C.parapsilosis*, *C. krusei*, *C.kefyr* and *C. glabrata*.

DISCUSSION

In the present study of 100 HIV seropositive patients with clinically suspected oral candidiasis attending ART centre of PMCH, Patna were selected randomly. With informed consent and brief history samples were obtained. Gram stain and KOH mount was done and inoculated on SDA media. Subjected to germ tube test, corn meal agar morphology, fermentation and assimilation test. The CD4 count of each patient was obtained. Age wise distribution-These findings were consistent with NACO data and other studies of Jabra Rizk *et al* (2001), Vargas KG *et al* 2002 and Anupriya *et al* (2007) with mean age of 37,38 and 34 respectively. Sex distribution- Male: Female ratio in this study is 1.17:1 as in the study by Ranganathan K *et al* 2008. Male: Female ratio being 1.03:1 and Enwuru CD *et al* 2008, 1.54:1. Where as study by Anupriya *et al* 2007 showed M:F ratio being 4.8:1. Male patients are more commonly diseased.

Mean CD4 count of study group:

Studies	Range	Mean CD4 Count
Fichtenbaum et al. (1997)		223 cells/ μ l
Cartledge et al. (1999)	2-230 cells/ μ l	15
Lattif et al. (2004)	42-200cells/ μ l	132+60.45
Anupriya et al. (2007)	66-294cells/ μ l	145+-52.1
Present study	11-347cells/ μ l	125+-78.45

Gram stain

Studies	Isolates with yeast cell	Sensitivity
Singh et al. (1999)	70 of 132 isolates	53%
Enwuru et al. (2008)	75 of 100 isolates	75%
Present	76 of 100 isolates	76%

Growth on SDA

Studies	No of cases	% growth on SDA (no of isolates)
Lattiff et al. (2004)	125	75%(95)
Schmidt et al. (2004)	121	100%(121)
Nadagir et al. (2008)	132	100%(132)
Enwuru et al. (2008)	213	34.7%(74)
Present study	100	100%(113)

Comparison of species isolated in various studies

Studies	% of Candida albicans	% of non-Candida albicans
Lattiff et al. (2004)	86%	14%
Patel et al. (2006)	78.6%	21.4%
Anupriya et al. (2007)	59.3%	40.7%
Chalcombe (2007)	86%	14%
Ranganathan et al. (2008)	85%	15%
Nadagir et al. (2008)	66.6%	33.3%
Enwuru et al. (2008)	40.5%	59.5%
Present study	28.31%	71.69%

Conclusion

In the present study of 100 HIV seropositive patients with oral candidiasis, we saw there was increase in incidence of non-candida albicans species compared to candida species. Oral Candidiasis also affects the ART and nutritional intake

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