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Prevalence of Oropharyngeal Candidiasis among Diabetic Patients and Their Speciation in a Tertiary Care Hospital

Lalitha S[1], Lucy Nirmal Medona M[2*]
[1]Govt Madurai Medical College Tamil Nadu, India
[2]Govt Thoothukudi Medical College Tamil Nadu, India

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***Corresponding author**
Lucy Nirmal Medona M

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Abstract: *C. albicans* is a normal human commensal, and the most common opportunistic fungal pathogen in immunocompromised patients**.** Colonization of the oropharynx and/or the alimentary tract often precedes invasive yeast infections. In immunosuppressed, neutropenic patients candidemia can result in widely disseminated disease usually with a fatal outcome if untreated. Certain non-albicans species such as *C. glabrata* and *C. krusei* are less susceptible to fluconazole than *C.albicans*. Non-albicans isolates are more likely to require higher doses of fluconazole to achieve clinical cure and are more frequently associated with severe symptoms(1). To study the prevalence of oropharyngeal candidiasis (OPC) in uncontrolled diabetic patients and identify the associated candida species. Prospective study carried out in (uncontrolled diabetic patients with fasting blood glucose level >126mg/dl for more than a year) the diabetic outpatient department in a tertiary care hospital for a period of 6 months.A total of 112 diabetic patients were included in the study and evaluated for the occurrence of candidiasis. Patients were screened for the presence of white patch on the tongue, mucosa of the hard and soft palate, and the buccal cavity. Two oral swabs were taken from the lesions, one for direct smear and the other for culture. Identification and speciation of the yeast isolates were done asper standard reference methods. Primary isolation on Sabouraud dextrose agar (SDA) followed by subculture on Cormeal agar with Tween 80(CMA-T80) and CHRO Magar Candida (CAC). The morphology of the blastoconidia, chlamydospores and the ability to produce pseudophyphae on CMA-T80 helps in the identification of the species. CAC is a selective medium for the isolation, identification and direct differentiation of several Candida spp. Of the 112-study population, the occurrence of oropharyngeal candidiasis (OPC) was (69.7%). C. tropicalis was the predominant species in the diabetics. The incidence was more in the age group >40 years in diabetics.

Keywords: Oropharyngeal candidiasis, immunocompromised, diabetics, Sabouraud dextrose agar (SDA), Cormeal agar with Tween 80(CMA-T80), CHRO Magar Candida (CAC).

INTRODUCTION

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. This increase is directly related to the growing population of immunocompromised individuals, resulting from changes in medical practice such as the use of intensive chemotherapy, radiotherapy and immunosuppressive drugs. Most superficial and subcutaneous fungal infections are easily diagnosed and readily amenable to treatment. Systemic infections can be life threatening and are associated with high morbidity and mortality.

The most frequently encountered pathogens are *Candida albicans* and Aspergillus speices. *Candida* spp. are considered opportunistic pathogens because they are usually benign colonizers of mucosal surfaces and become pathogens in situations in which the host resistance to infection is lowered locally or systemically. Species commonly implicated in human infections are, C.albicans, C.glabrata, C.guilliermondii, C..krusei, C.lusitaniae, C.parapsilosis, C.tropicalis[2].

The forms of disease most commonly caused by *Candida* spp. involve the female genitalia, the skin and nails, and the oral cavity, sometimes with concomitant oesophageal invasion. *Candida* infection of deep tissues are almost always, the result of hematogenous spread from an endogenous or less often an exogenous site. In immunosuppressed patients, particularly with severe neutropenia candidemia can

result in widely disseminated disease usually with a fatal outcome if untreated.

This prospective study was conducted to determine the prevalence of oropharyngeal candidiasis among diabetic patients and their speciation.

MATERIALS AND METHODS

The study group included the patients attending the diabetic outpatient department in a tertiary care hospital for a period of 6 months.

Inclusion criteria

Uncontrolled diabetic patients whose fasting blood glucose level was >126mg/dl for more than a year (American Diabetic Associations Diagnostic Criteria)

Exclusion criteria

Patients with known history of diabetes for a short period (6 months) whose fasting blood glucose level was <126mg/dl. A total of 112 diabetic patients were included in the study and evaluated for the occurrence of candidiasis. Speciation of the yeast isolates were done.

Specimen collection

History was elicited regarding the risk factors like smoking, alcoholism and tobacco chewing. Patients were screened for the presence of white patch on the tongue, mucosa of the hard and soft palate, and the buccal cavity. Two oral swabs were taken from the lesions using sterile Himedia dry cotton swabs, one for direct smear and the other for culture.

Direct examination

Grams stain was performed on smears prepared from the oral swab of all the 112 samples and screened for budding yeast cells. Presence of gram positive, oval budding yeast cells (2-4µm), elongated filamentous cells, connected in a sausage like manner (pseudohyphae) or as truly septate hyphae were consistent with the morphology of Candida species.

Primary isolation on sabouraud dextrose agar (SDA)

Oral swabs freshly taken were cultured onto Sabouraud dextrose agar (pH5.6) containing chloramphenical (50mg/l) and gentamicin (20mg/l) to minimize bacterial contamination. Cycloheximide was not incorporated in isolation media, because it inhibits the growth of some species (*C.tropicalis, C.krusei, and C.parapsilosis*). The inoculated SDA plates were incubated at 37°C for 48-72 hrs. Relatively large, butyrous colonies were easy to distinguish and isolated colonies from each positive culture were selected and again sub cultured to SDA plates for stock cultures.

C.albicans was identified by Germ tube test, production of chlamydospores in Corn Meal-Tween 80 agar and CHROMagar Candida medium.

Germ tube test

A small portion of isolated colony of yeast was suspended in a sterile test tube containing 0.5 ml of pooled human serum and incubated at 37^{o}C for two hours. A drop of yeast serum suspension was placed on a microscopic slide, overlaid with a cover slip and examined microscopically for the presence of germ tube. The test is not valid if examined after 2 hrs. Filamentous extension from the yeast cells about ½ the width, and 3-4 times the length of the cell, with no constriction at the point of origin was considered positive.

Cormeal agar with Tween 80

Cornmeal 2gm, agar 15gm and Tween 80 -7ml were added to 1 liter of distilled water, pH adjusted to 6.2 and autoclaved at 121°c, 15lbs for 15 mins. Medium was cooled to 45-50°c, poured into sterile Petri dishes and allowed to solidify for atleast 30 mins. Tween 80 was added to corn meal agar to reduce the surface tension and enhance the formation of hyphae and blastoconidia. The morphology of the blastoconidia, chlamydospores and the ability to produce pseudophyphae helped in the identification of the species**.** (Rippon. JW Medical mycology)

Procedure

Using an inoculating needle, a visible paste of the organism was obtained. The needle was drawn through the agar making two perpendicular lines in the shape of an 'x**'.** A cover slip was flamed, allowed to cool and placed over the central area of the 'x' in order to reduce the O_2 tension. Reduced O_2 tension stimulated the chlamydospore production. The plate was sealed with a tape and incubated aerobically at 25-30°c for upto 72hours.Inverted plates were examined microscopically using a low power objective, along the edge of the coverslip for detection of chlamydospores. (Koneman, Color Atlas and Diagnostic Microbiology 5th edition pg 1043 & Hardy and Hugo diagnostics)

Interpretation

C.albicans produce compact clusters of blastoconidia at regular intervals along pesudohyphae. C.dubliniensi produce clusters of chlamydospores usually in doublets or triplets.C.tropicalis produce abundant pseudomycelium composed of elongated blastoconidia widely spaced, in single or small clusters along the hyphae. C. parapsilosis produce thin, much branching pseudomycelium and verticals of few ovoid to elongate blastoconidia. C.krusei produce elongated blastoconidia in tree or crossed match stick like arrangements.

C.glabrata produce no pseudomycelium, but small round yeast cells were seen.

C. kefyr produce abundant pseudo mycelium with very elongated blastoconidia, which fell apart, parallel like logs in a stream.

CHROMagar Candida (CAC) is a selective medium for the isolation of fungi that simultaneously provides direct differentiation and identification of several Candida spp. A suspension of yeast was made from an overnight culture on Sabouraud dextrose agar and inoculated to CAC. The plates were incubated in atmospheric air at 37ºC as recommended by manufactures. All cultures were read at 48 hrs. Colonies of *C.ablicans* and *C.dubliniensis* appeared lighter and darker green, respectively. *C.tropicalis* appeared with a blue green hue and *C. krusei* colonies light pink and dry with a light border. Other yeasts appeared cream coloured.

RESULTS

A total of 112 oropharyngeal (OPC) swabs were collected from individuals who had uncontrolled diabetes mellitus for more than a year. Of the 112-study population, 71(63.4%) were male and 41(36.6%)female.

Table-1: Demographic character of the study population

Age	M	F	Total
< 20 years	2	2	4(3.6%)
21-30	4	3	7(6.3%)
31-40	13	9	22(19.6%)
41-50	17	10	27(24.1%)
51-60	20	5	25(22.3%)
>60	23	4	27(24.1%)
Total	79	33	112

Primary isolation of candida species from clinical samples

When 112 oropharyngeal (OPC) swabs were examined by Grams staining and cultured on Sabouraud dextrose agar, 78 (69.6%) showed budding yeast cells and 27 (32.1%) showed pseudohyphae on direct examination and creamy growth on SDA.

Rapid identification of candida albicans

Germ tube test

To confirm the identity as C.albicans the germ tube test was performed for all the 78 *Candida* isolates. Of the 78 isolates 41 (52.6%) showed the presence of germ tube and 37 (47.4%) showed no evidence of germ tube.

Morphology / Chlamydospore production on Cornmeal Tween80 medium(CMA-T80)

All the 78 *Candida* isolates from SDA were subcultured on to Cornmeal Tween80 agar for identifying the morphology and arrangement of the blastoconidia, chlamydoconidia, pseudohyphae and hyphal structures. 43 (55.1%) isolates produced chlamydospores and 35(44.9%) showed no chlamydospores.

CHROMagar Candida

The chromogenic substrate incorporated in the CHROMagar *Candida*, aided in the speciation of *Candida* on the basis of the specific colony color, in particular for the C.albicans (light green), C.dubliniensis (dark green), C.tropicalis (blue green hue) and C.krusei (light pink to white) and other species (white). 41(52.6%) isolates were light green, 2(2.6%) were dark green, 17 (21.8%) were blue green hue and 18 (23%) were white in color.

Table-2: Isolation of Candida in CHROM agar Candida media

Colour	No. of positive Isolates	Species
Light Green	41 (52.6%)	C.albicans
Dark Green	2 (2.6%)	C. dubliniensis
Blue Green	17 (21.8%)	C. tropicalis
White	18 (23%)	Others
Total	78	

Distribution of Candida species among the clinical isolates

Together with all the identification tests the **78** isolates were speciated as 16 (20.5%)as *C.albicans*, 24 (30.8%)as *C.tropicalis*, 12 (15.4%)as *C.parapsilosis*, 4 (5.1%)as *C.kefyr*, 8(10.6%) as *C.glabrata*, 6(7.7%)as *C.guilliermondii*, 4(5.1%)as *C.krusei*, 2(2.6%)as *C.dubliniensis*, 2(2.6%)as *C. lusitaniae*.

Table-3: Distribution of Candida species among the clinical isolates

Species	Male	Female	Total
C. tropicalis	17 (70.8%)	7 (29.2%)	24 (30.8%)
C. albicans	9 (56.25%)	7 (43.75%)	16 (20.5%)
C. parapsilosis	9	3	12 (15.4%)
C. kefyr	3	1	4 (5.1%)
C. glabrata	4	4	8(10.6%)
C. guilliermondii	4	2	6(7.7%)
C. krusei	2	2	4(5.1%)
C. dubliniensis	2	0	2(2.6%)
C. lusitaniae	2	0	2(2.6%)
Total	52(66.7%)	26(33.3%)	78

C. tropicalis (30.8%) was the predominant species. C.albicans constituted 20.5% and non-albicans 79.5%. C.tropicalis and Non- albicans were predominantly isolated from the male population.

Age wise distribution of Candida species

The isolation of *C.albicans* was more in the age group 31-40years (50%) %), and that of non-*albicans* in the age group 51-60years (33.3%). The 2 *C. dubliniensis* were isolated from the age >60years.

Table-5: Age wise distribution of Candida species

Species	Below 20	21-30	31-40	41- 50	51- 60	Above 60	Total
C.albicans	2	0	8(50%)	2	2	2	16
C.tropicalis	0	1	2	5	10	6	24
C.parapsilosis	0	1	1	4	4	2	12
C.kefyr	0	0	1	1	2	0	4
C.glabrata	0	0	2	0	4	2	8
C.guilliermondii	0	0	1	0	2	3	6
C.krusei	1	0	0	0	2	1	4
C.dubliniensis	0	0	0	0	0	2	2
C.lusitaniae	0	0	0	0	0	2	2
Total	3	2	16	12	26(33.3)	20	78

DISCUSSION

Although *C.albicans* remains the most common species encountered as a cause of human infection, other *Candida* species have been increasingly associated with disseminated disease since the 1990. Since candidemia is associated with a high mortality rate, prompt appropriate antifungal therapy is essential in the immunocompromised.

A total of 112 clinical samples were collected from the Diabetic Outpatient Department of a tertiary care centre for a period of 6 months to study the prevalence of oropharyngeal candidiasis (OPC) and identify candida species.

In the present study, among the112 study population, the occurrence of oropharyngeal candidiasis (OPC) was (69.6%) which is in favour of Knight. l *et al*. [3] who demonstrated that diabetics are more susceptible to candidiasis with an incidence of 67%. This is also supported by Vazquez, J. A., and J. D. Sobel *et al*. 1995[4]. Observation of OPC mostly in diabetics (71%) who have poorly controlled blood sugar levels. In this study, the incidence of oropharyngeal candidiasis (OPC) was 52(66.7%) in male and 26(33.3%) in female. The high incidence in male was associated with the habit of smoking and alcoholism in males. When the age factor was taken into consideration, the incidence was more in the age group >40 years in diabetics. This is in accordance with Benito Almirante & Dolrs Rodriguez *et al*.[5] a population based surveillance were the age specific incidence rate was highest in infants (38.8 cases100,000 population)/and in those aged > 65 years (12cases/100,000 population) for blood stream *Candida* infections.

CONCLUSION

The yeast *Candida* was commonly isolated from the oropharynx of the immunocompromised individuals. The incidence of oropharyngeal candidiasis is high among the diabetics. C. tropicalis was the predominant species in the diabetics.

The males were more affected due to the associated risk factors and the age group above 40yrs are at high risk of developing oropharyngeal candidiasis.

Direct microscopy using Grams stain and primary isolation on Sabourauds dextrose agar was the most simple, economic and easy method for the isolation of the yeast *Candida*.

The early identification of C. albicans was by, germ tube test, production of chlamydospores on the Corn meal Tween 80,specific colour of the yeast colonies on the CHROMagar *Candida* Medium. The confirmation of the *Candida* species was by the morphology and arrangement of the blastoconidia, pseudohyphae and hyphal structures on the semi starvation medium Corn Meal Agar with Tween 80. And this can be further confirmed by Carbohydrate Assimilation pattern on the carbohydrate free, Yeast nitrogen base medium.

SUGGESTION

As *C. albicans* is a normal human commensal, it is not surprising that this organism is the most common opportunistic fungal pathogen in immunocompromised patients[6]. Colonization of the oropharynx and/or the alimentary tract often precedes invasive yeast infections[7].

The oral manifestations of oropharyngeal infection in immunocompromised patients present a particular challenge for both medical and dental professionals because clinical signs and symptoms may be minimal and accurate diagnosis and appropriate treatment may be difficult. Effective control of infection and management of oral symptoms are important and this is achieved by the judicious use of topical and systemic agents and by maintaining good oral hygiene. Therefore the isolation, identification and speciation of *Candida* from clinical specimens are very essential in deciding the antifungal of choice.

More recent data, suggest that in the setting of extreme immune debilitation, particularly neutropenia and prematurity, a change in epidemiology of candidiasis has occurred with a reduction in the rates of C.albicans in favour of the non albicans speices. in particular C.glabrata, *C..Krusei*, C.parapsilosis and C.tropicalis[8]**.** Whether these changes are a consequence of increased immuno suppression, the use of prophylactic antifungal treatments or absence of adequate infection control measures is uncertain. In general, routine prophylaxis of mucosal candidiasis, particularly OPC, has been discouraged[9].

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