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Evaluating the Prevalence of Candida Species in the Oral Cavity of Immunocompromised Patients

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Abstract: ***Candida species are normal commensal of the oral microbial flora and get established there during or soon after birth. Lesions of thrush are seen in children and adults of all ages whenever the number of candida in the oral cavity increases significantly . During the past two decades, there has been a significant increase in the prevalence of fungal infections caused by Candida species. The yeast candida being the main cause of candidiasis is commonly isolated pathogen from immunocompromised patients. The trend in the resistance acquired by some species of candida leads to the importance of identification to the species level in immunocompromised patients. In present study we have selected 50 subjects (male and female) who are HIV positive(10),diabetes mellitus(10),vitamin deficiencies(10),radiotherapy patients(10) and the patients on broad spectrum antibiotics(10). Oral samples were collected with the help of sterile cotton swabs, and inoculated on sabouraud`s dextrose agar (SDA) for isolation. After appearance of the colonies on SDA, individual colony is tested for identification of different species of candida on the basis of biochemical and assimilation test. The prevalence of oral candidosis and frequency of isolation of Candida species and its distribution were determine statistically by using x^2 test ,it is found that difference is not statistical significant between Candida albicans and Candida non albicans among the sex, it can be present in target population.***

Keywords: Immunocompromised, prevalence, SDA, *Candida albicans* and *Candida nonalbicans*.

1. Introduction

Among the several hundred species of microorganisms in the oral cavity, yeasts, especially members of the genus *Candida*, are representative of the few fungi considered to be commensal oral flora. *Candida albicans* is the most common species isolated from the human oral cavity, while other species such as *C. glabrata, C. tropicalis, and C. dubliniensis*, are less frequently found(.1,2) The term *candida* originate from Latin word candid ,meaning white (3). *C albicans* is most commonly found in thrush (4). Oral thrush is a superficial infection of the mucous membrane characterized by white adherent patches of pseudomycelium which frequently involves lesions, sores, fissures and ulcers in the mouth which could either be acute or chronic (5,6) Oral candidiasis is a sign of impaired local or systemic defence mechanisms. Reduced saliva secretion, deficiencies of humoral or cell-mediated immunity, local mucosal diseases and use of wide spectrum antibiotics are predisposing factors (7). The use of broad – spectrum antibiotics, steroids, or other immunosuppressive agents, diabetes mellitus cancer patients and organ transplantation can increase the risk for candidal infections (8). The prevalence of oral candidiasis in various countries varies among studies according to location, age of the patients, and the site sample, and has been reported to range from 20- 75% (9). Oral candidiasis, which is frequently caused by *Candida albicans*, is one of the most common fungal opportunistic infections in immunocompromised patients (10).Patients with candidiasis may display various symptoms including burning, painful sensation, change of taste, and swallowing difficulty, but most often are asymptomatic (11) The mechanisms that protect the human host against fungal infection depend upon a combination of factors. In the immunocompromised patient, alterations in phagocytic or lymphocytic cell numbers or function are often the most critical factors predisposing to fungal infection(12) .The purpose of the present study is to evaluate the prevalence of *candida* species in the oral cavity of immunocompromised patients to determine the treatment for the infection and minimized the treatment failure or recurrent infection. In present study we have selected 50 subjects (male and female). Oral samples are collected and inoculated on sabouraud`s dextrose agar (SDA) for isolation. After appearance of the colonies on SDA, individual colony is tested for identification of different species of *candida* on the basis of biochemical and assimilation test.The prevalence of oral candidosis and frequency of isolation of *Candida* species and its distribution determine statistically by using x^2 test.

2. Materials and Methods

50 subjects were involved in this study. The subject selected were HIV positive (10), diabetes mellitus (10), vitamin deficiencies (10), radiotherapy patients (10) and the patients on broad spectrum antibiotics (10) of having thrush, angular chelitis, and denture stomatitis. The medical history of each individual was checked for factors known to affect oral cavity and can cause oral candidiasis .The conventional methods of identifying yeast to species level in the clinical microbiology laboratory rely on criteria such as morphology, growth characteristics and carbon source assimilation or fermentation as well as appearance on differential isolation media (6,13) Isolates of *C. albicans* are typically identified by their ability to form germ tube (GT) or chlamydospores under the appropriate conditions(14).

***Sample Collection*:** Specimens were collected from patients with all sign and symptoms with oral disease, oral hygiene, tobacco use, orthodontics appliances or denture wearing and medication were noted. Specimens from oral lesions were collected by passing sterile cotton swab several time across the surface, including oral thrush. Immediately after

sampling, each swab was replaced in sterile tube and transported within 30 minutes of sampling from the place of collection of the laboratory (15).

***Microscopic Examination*:** *Candida* infection is diagnosed by presence of budding yeast with hyphae and pseudohyphae on KOH (potassium hydroxide) examination (16). A10% or 15% KOH solution can be used, which acts as clearing agents of the tissues and cellular debris but does not damage the fungal cells. Specifically, the KOH digests proteinous debris, bleaches pigment, and dissolves the "cement" that hold keratinized cells together (17).Samples were processed by standard method .Direct smear were prepared and Gram staining was done to look for Gram positive budding yeast cells and pseudohyphae (18).

***Isolation*:** The swab is inoculated on sabouraud's dextrose agar slopes and incubated at 37ºC for 24 hours to 48 hours. Creamy white colored colonies shows presence of *Candida* .Macroscopic (creamy white colonies) and microscopic (yeast cell pseudohyphae and blastospores) examination of the growth verified by diagnosis of candidiasis. The isolated colonies are tested for identification of species (19).

***Identification*:** The tests are performed on corn meal agar for chlamydospores and germ tube formation. The isolated colonies are subculture on corn meal agar plate and incubated at 25ºC for 48-72 hrs. *Candida albicans* species was identified microscopically by production of chlamydospores (20). The germ tube test to identify *candida albicans* was done according to the method of cheesbrough. About 0.5ml of human blood serum was poured into small tube and each isolate were inoculated into tube using sterile wire loop. Incubation was in a water bath for 2-4 hrs. A drop of serum yeast culture was transferred onto a glass slide using a Pasteur pipette and covered with a cover slip. Examination for germ tubes or blastospores was done under the 40X objective. The presence of sprouting yeast cells was a positive identification for C. albicans (20).Germ tube negative and chlamydospore negative species are further identified by biochemical and assimilation test for different non *candida* albicans species.

Statistical work*:* In this study, a chi-squared test ($\chi 2$) was employed to determine the statistical significance of data.
Results: A total number of 50 isolates were analyzed in this study. These organisms were fresh isolates, all of which were cultured from specimens.
Sample collection: The isolates were from oral thrush of patients suffering from oral candidiasis (Fig.1).

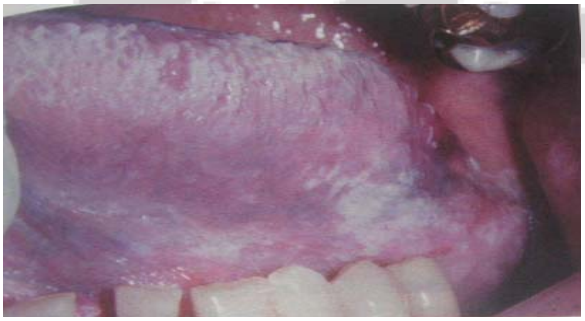

Figure 1: Shows Oral Thrush of Patient

Microscopic identification:10%KOH preparation shows yeast cell with pseudohyphae. Gram staining technique was used as a preliminary test prior identification just to ensure that the archived *candida* species were still viable and free of contamination .Gram staining shows Gram positive yeast cells with pseudohyphae (Fig 2).

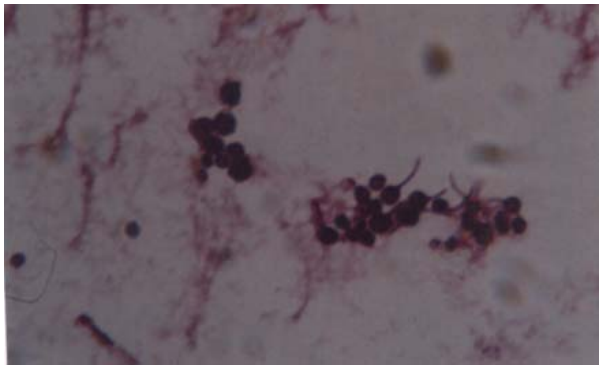

Figure 2: Shows Gram positive yeast cell with pseudohyphae

Cultural characteristics: Culture on SDA shows pure colonies which are creamy white colour with yeasty odour (Fig 3). Different species (Spp) of *candida* isolated among male and female (Table no.1.with graphical representation). All candida albicans shows germ tube formation and also they form chlamydospores on corn meal agar.

Table 1: Different spp.of candida among both sex

Sex	*C.albicans*	*Non.C.albicans*	*Negative*
Male	20	3	2
female	23	1	1
Total	43	4	3

Graphical representation of Different Species of *Candida* Isolated among male and female in immunocompromised patients

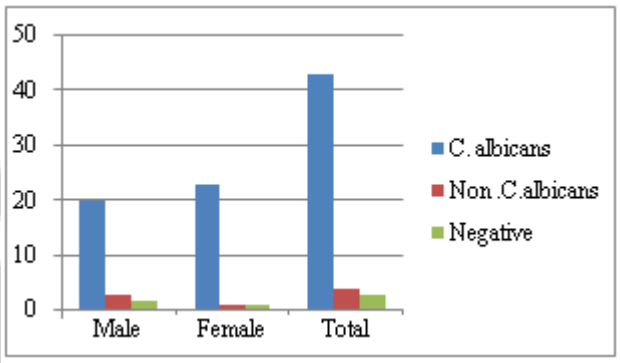

Figure 3: Shows Creamy white colour colonies on SDA

Biochemical reaction: Candida species utilized glucose ,galactose, sucrose and maltose. All the non albicans *candida* utilized starch; *Candida albicans* utilized raffinose in addition to the four sugars in common .

According to the results obtained, *candida albicans* was the, most frequently isolated with 86%,this was followed by *C. tropicalis* 4%, *C. krusie* 2% and *C.parapsilosis* 2%. Calculating the data statistically by using, a chi-squared test ($\chi 2$) it is found that difference is not statistical significant between *Candida albicans* and *Candida non albicans* among the sex (Table no.1),it can be present in target population. Poor oral hygiene, tobacco smoking and the use of oral contraceptive pills were recorded as possible risk factors for development of the oral lesions. Predisposing factor for infection were identified which included hematological malignancies, complications of uncontrolled diabetes mellitus. Most of these patients were receiving systemic antibiotics. Other medications included cancer chemotherapy systemic corticosteroids, fluconazole and irradiation therapy. Poor nutritional status was observed in 70% of the patients. Thus, lastly it can be said that isolation and screening of pathogenic yeast is necessary for evaluating the prevalence of fungal diseases in oral cavity of immunocompromised patients and to identify its species, genus and can determine the treatment for the infection and minimize treatment failure or recurrent infection.

3. Discussion

Infections caused by *Candida* are on the rise as the number of immunocompromised patients in the community increases. Thus, oral candidiasis is the most common oral opportunistic infection in immunocom-promised patients(2).Intensive chemotherapy, high doses of oral and systemic corticosteroids, potent antibiotics and underlying diseases such as diabetes mellitus, neutropenia, and xerostomia have all contributed to this phenomenon(21). Studies have shown that this organism can account for up to 75% of the yeasts recovered from sites of infection(22).Rapid identification of candidiasis is important for the clinical management of immunocompromised patients (23).The total numbers of 50 isolates were studies ,47 *candida* species were isolated from oral cavity. *Candida albicans* was found to be the predominant with 86% followed by *C. krusi* 2% *C. tropicalis* 4% *C. parapsilosis* 2%. In agreement with findings of others (Back-Brito *et al.,* 2009; Williams and Lewis,(2000), the majority of yeast isolates from oral cavity swabs were *C. albicans* 70%, Germain *et al.,* (2001) found the distribution of Candida species to be as follows: *C. albicans* 54%, *C.glabrata* 15%,*C.parapsilosis* 12%, *C.tropicalis* 9% and *C.krusei* 3%. Raju and Rajappa (2011) reported a similar pattern of distribution of species. The findings of the present study are more or less similar with the previous study. This could be due to variation in geographical distribution of various *Candida* species. In the present study, the isolation rates of *Candida* species is high in ages ranging from 41-80 years old (Table no.2).

Table 2: Age distribution among which *candida* species isolated

Age(years)	*No. of isolates*	*% of isolates*
Below 40	15	30%
Above 40	35	70%
Total	50	100%

The similar studies were conducted by Pinho (2002) and Zaremba *et al.* (2006), and found the isolation rates of *Candida* species to be high in ages ranging from 60-80 years.

4. Conclusion

In the present study, we demonstrated that *Candida albicans* was the most common species associated with oral carriage in immunocompromised patients. The prevalence of oral *candidosis* and frequency of isolation of *Candida* species and its distribution were determine statistically by using x^2 test ,it is found that difference is not statistical significant between *Candida albicans* and *Candida non albicans* among the sex. The frequent occurrence of *Candida albicans* in oral cavity of immunocompromised indicates a need for effective management of the infection prior to any treatment, as severe complications can otherwise result. Present study suggests that routine checks for opportunistic infections including oral candidiasis are important and should be carried out at intervals to help monitor disease progression and also prevent subsequent complications such as candidemia. Identifying *Candida* to its species level is important because it helps guiding proper treatment.

References

[1] Samaranayake LP(1991). Superficial oral fungal infections. Curr Opin Dent.1:415-22.
[2] Scully C, El-Kabir M, Samaranayake LP.(1994) Candida and oral candidosis: a review. Crit Rev Oral Biol Med.; 5:125-158.
[3] S. L. Zunt (2000), “Oral candidiasis: diagnosis and treartment,” The Journal of Practical
[4] Hygiene, 9, 31-36.
[5] Burkets Oral Medicine, diagnosis and treatment: J. B. Lippincott Company Philadelphia: IX edition :63.
[6] Elias Anaissie, Gerald P. Bodey, Hagop Kantarjian, Cynthia David, Kyle Barnett,et-al. (1991). Fluconazole therapy for chronic disseminated candidiasis in patients with leukemia and prior amphotercin B therapy .Am.J. Med, 91:(2):142-150
[7] Odds F., Bernaerts R. (1994), CHROM agar *Candida,* a new differential medium forpresumptive identification of clinically important *Candida* species. J Clin Microbiol, 32: 1923-29.
[8] R. Rautemaa, P. Rusanen, M., Richardson, J. H. Meurman (2006), Optimal sampling site for mucosal

candidosis in oral cancer patients is the labial sulcus.J.Med Microbiol, 55, 1447.
[9] Daniluk T, Tokajuk G, Stokowska W,Fiedoruk K, Sciepuk M, Zaremba ML, Ro汐kiewicz D, Cylwik-Rokicka D, Kedra BA, Anielska I, Gorska M, Kedra B (2006).Occurrence rate of oral Candida albicans in denture wearer patients. Advances in Medical Sciences: 51.(suppl.1).77-80.
[10] Hana M, Khaled H, Ali Z, and Mawieh H (2004).Isolation and characterization of Candida spp. In Jordanian Patients: Prevalence, Pathogenic Determinants, and antifungal sensitivity. Japanese. J. Infect. Dis. 57, 279-284.
[11] Klein RS, Harris CA, Small B, Moll B, Lesser M, Friedland GH (1984) Oral candidiasis in high-risk patients as the initial manifestations of the acquired Immunodeficiency syndrome. Natl. Eng. J. Med. 9: 354-358.
[12] Samaranayake LP. Host factors and oral candidiasis.In: MacFarlane TW, Samaranayake LP, editors(1990). Oral candidosis. London: Butterworth & Company Ltd .66-103.
[13] Warnock DW. Introduction to the management of fungal infection in the compromised patient. *In:* Warnock DW, Richardson MD,(1991) eds. *Fungal infection in the compromised patient*, 2nd ed. Chichester: John Wiley; 23-53
[14] Goldschmidt M.,Fung D., Grant R., White J.,Brown T. (1991), New aniline blue dye medium for rapid identification and isolation of *Candida albicans*. J Clin Microbiol 29: 1095-99.
[15] Berardinelli S., Opheim DJ. (1985), New germ tube induction medium for the identification of *Candida albicans*. J Clin Microbiol, 22: 861-62
[16] Ozlem OSMANAÚAOÚLU1 Nurten ALTINLAR2 Sefa C. SA.ILIK3 Cumhur..KM.3 Ahmet AKIN (2000), Identification of Different *Candida* Species Isolated in Various Hospitals in Ankara by Fungichrom Test Kit and Their Differentiation by SDS-PAGE Turk J Med Sci, 30,.355-358.
[17] C. Baveja, "Medical mycology,"(2010) in *Text Book of Microbiology for Dental Students*, 322-323.
[18] JoAnn Parker Fenn (2007), Update of medically Important Yeasts and a Practical Approach to Their Identification. Labmedicine, 38:(3): 178-183.
[19] Jawetz, Melnick, Adelberg: Text book of Medical Microbiology Publisher Mc Graw Hill Medical:24th Edition: Chapter 45:632-43.
[20] Shaheen M. A. and Taha M. (2006), Species Identification of candida Isolates obtained from oral lesions
[21] of Hospitalized and Non Hospitalized patients with oral candidiasis, Egyptian Dermatology Online Journal: 2. (14).1-14.
[22] Cheebrough, M. (2000), Fungal Pathogens. In:Distrtict Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press.Cambridge, UK.244.
[23] Garber GE(1994). Treatment of oral *Candida* mucositis infections. *Drugs*;47:734-40.
[24] Espinel-Ingroff A, Brachiesi F, Hazen KC, Martinez-Suarez JV & Scalise G(1998). Standardization of antifungalsusceptibility testing and clinical relevance. Med Mycol, 36: 68-78
[25] Mousavi SAA, Salari S, Rezaie S, Nejad NS, Hadizadeh S,Kamyabi H & Aghasi H(2012). Identification of Candida species isolated from oral colonization in Iranian HIV-positive patients, by PCR-RFLP method. Jundishapur J Microbiol 5(1): 336-340. DOI: 10.5812.
[26] Back-Brito, G.N., Mota, A.J., Vasconcellos,T.C., Querido, S.M.R., Jorge, A.O.C., Reis,A.S.M. and Balducci Cristiane,Y (2009).Frequency of *Candida* spp. in the OralCavity of Brazilian HIV-Positive Patientsand Correlation with CD4 Cell Counts and Viral.Load. *Mycopathol.,* 167: 81-87.
[27] Germain, G., Laverdiere, M. and Pelletier R(2001). Prevalence and antifungal susceptibility of 442 *Candida* isolates from blood and other normally sterile sites: Results of a 2- year (1996-1998) multicentresurveillance study in Quebec, Canada. J Clin Microbiology. 39: 949-953.
[28] Raju, S.B. and Rajappa, S.(2011). Isolation and Identification of Candida from the OralCavity. *ISRN Dentistry*,Volume 2011,Article ID 487921, 7 pages. doi: 5402/2011/487921.
[29] Zaremba,M.L.,Rokicka,D.C.D.,Kierklo,A.and Tokajuk,G .,(2006). Incidence rate of *Candida* species in the oral cavity of middleaged and elderly subjects. *Advances medical science: 51*.

Author Profile

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