Oral carriage of candidiasis in patients with oral dental diseases: predisposing factors, species and their antifungal susceptibility patterns

Abstract

The aim of this study was to evaluate prevalence, frequency and predisposing factors as well as their antifungal susceptibility pattern of oral candidiasis (OC) in patients attending outpatient’s dental clinic with various dental diseases. A total of 150 patients were enrolled in this study aged from 4 to 73 years attended outpatient dental clinic, at Zahra district Tripoli, Libya between May 20017 and May 2018. The purpose of this study was to evaluate the frequency of yeasts in the oral cavity, and to determine the main yeast species present, their correlation with various dental problems as well as other predisposing factors and their antifungal susceptibility patterns. One hundred and fifty patients were enrolled in this study. Patients were submitted to an odontological examination for the identification of dental caries and dental plaque, and other dental problems, as well as for yeast culture. Identification of all species was based on standard mycological methods and antifungal susceptibility test was performed by using disk diffusion susceptibility method and several antifungal drugs such as (fluconazole, amphotericin B, ketoconazole, econazole, itraconazole) were used. The frequency oral carriage of yeasts was 64 % (96), with 78% aged from 20 to 60 years old. Caries, plaque, gingivitis periodontitis, and lower teeth are the most dental problem in which, more than 50% harbors candida species in their oral cavity. Diabetes, pregnancy and smoking, is among common predisposing factors. Candida albicans was the most prevalent species (41.7%), C. glabrata (27.1%) and C. dubliniensis (11.5%). Susceptibility test show that Candida albicans was highly resistant to most azole antifungal and Candida dublinskiensis was highly resistant to fluocytosine. Other candida species show variable susceptibility to various antifungal drugs.

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Material and methods: One hundred and fifty patients were enrolled in this study. Patients were submitted to an odontological examination for the identification of dental caries and dental plaque, and other dental problems, as well as for yeast culture. Identification of all species was based on standard mycological methods and antifungal susceptibility test was performed by using disk diffusion susceptibility method and several antifungal drugs such as (fluconazole, amphotericin B, ketoconazole, econazole, itraconazole) were used.

Results: The frequency oral carriage of yeasts was 64 % (96), with 78% aged from 20 to 60 years old. Caries, plaque, gingivitis periodontitis, and lower teeth are the most dental problem in which, more than 50% harbors candida species in their oral cavity. Diabetes, pregnancy and smoking, is among common predisposing factors. Candida albicans was the most prevalent species (41.7%), C. glabrata (27.1%) and C. dubliniensis (11.5%). Susceptibility test show that Candida albicans was highly resistant to most azole antifungal and Candida dubliniensis was highly resistant to fluocytosine. Other candida species show variable susceptibility to various antifungal drugs.

Conclusion: This finding indicated a correlation between the presence of caries and other dental diseases with yeasts carriage in the mouth. However, there was no clear cut correlation between yeasts carriage in healthy and health patients.

Keywords: candida species, gingivitis, prosthesis, diabetes
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Introduction

Different species of yeasts can colonize the oral mucosa under well-balanced ecosystem conditions. The primary etiological agent of oral candidiasis is the yeast *C. albicans*; however, other species that cause disease less commonly include *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, and *C. Dublinsiensis*.

However, alterations in this equilibrium may transform these microorganisms into infectious agents, expressing virulence factors and invading tissues. The incidence of fungal infections has increased in recent years in many countries, which has stimulated scientific studies. In relation to oral infections, such as stomatitis, candida has been found in different situations such as root canal infections, especially in the root canals of obturated teeth in which treatment has failed. *C. albicans* has been associated with cases of persistent root canal infections, because this yeast can be resistant to some intracanal medications.

Dental caries is a chronic and multifactorial disease that, although avoidable, still represents an important problem in public health, since it affects approximately 90% of the population, mainly children and adolescents, compromising their quality of life and development. The development of caries depends on the interaction of multiple factors relating to the host, especially a diet rich in fermentable carbohydrates, and the presence of Cariogenic microorganisms. Microbial process resulting from a nonspecific accumulation of acid-producing microorganisms on teeth; it should be interesting to confirm the possible involvement of other microorganisms in their formation. Classically, the microorganisms involved in the genesis and development of caries are bacteria such as Streptococcus mutans and other cocci and rods. Furthermore there is evidence of the involvement of *C. albicans* in the etiology of dental caries. Yeast cells are certainly acidogenic microorganisms, but the primary caries process has not yet been linked to the presence of yeast cells. Therefore, the aim of this study was to evaluate the frequency of yeasts in the oral cavity, determine the main species present, and assess the possibility of a correlation between the presence of yeasts with caries and dental plaque as well as other predisposing factors and their antifungal susceptibility patterns.

Materials and methods

Patients oral rinse study sample

One hundred fifty asymptomatic individuals patients with dental oral disease referred to the dental clinics in ZAHRA City were enrolled in this study. Oral rinse mouth samples were taken as well as sample from infected teeth when involved. A patient’s information sheet (Questionnaire survey) was used included patient information such as age, gender, oral condition, gingivitis, periodontitis, caries, smoking and any other predisposing factors.

Samples collections and processing

Samples were obtained by an oral rinse in which the mouth was washed for 30 second with 10mL of sterile water. The mouthwash liquid was deposited in conical tubes and stored in an insulated container until microbiological processing. Later, this suspension was washed three times in sterilized 0.1M phosphate-buffered saline pH 7.4 (PBS) by centrifugation 3.000rpm, the pellet was resuspended in 1mL of PBS, and 10μL aliquots were inoculated with a bacteriological loop onto the surface of Sabouraud’s dextrose agar with antibiotic (Oxoid Ltd). Plates were incubated at 30°C for 24 to 72h. Yeasts were identified by germ-tube production, micromorphology, and chlamydospores production on Tween 80-corneal agar. All isolates with germ tube test positive and chlamydospores positive on corn meal agar plus tween 80 were identified as *C. albicans* or *C. dublinsiensis*. Sunflower seed agar and xylose hypertonic media was used to differentiate *C. albicans* from *C. dublinsiensis*. Non candida albicans species were identified by API 20C AUX (bioMérieux’s).

Antifungal susceptibility testing

Five distinct colonies of approximately one mm from each 24 hours old culture grew on Sabouraud Dextrose Agar incubated at 35±2°C. Colonies were suspended in 5mL of sterile 0.85% Saline. The resulting suspension was adjusted to the turbidity to yield 1×10^6-5×10^10 cells/mL (i.e., 0.5 McFarland standard). A sterile cotton swab moistened with the inoculums suspension was used to apply to a 90mm diameter plate containing Mueller-Hinton agar supplemented with 2% glucose and 0.5μg/mL methylene blue (GM-MH agar medium). The plates were allowed to dry for 5-15 minutes before disks were placed in the center of the agar. The following antifungal discs was purchased from Liofilchem, Italy were used are Amphotericin B (20μg), Caspofungin (5μg), Clotrimazone (50μg), Econazole (10μg), Fluconazole (25μg and 100μg), Fluconazole (1μg), Itraconazole (50 μg), Ketoconazole (10μg), Miconazole (10μg), Nystatin (10-100 IU), Posaconazole (5μg), Voriconazole (1μg). The plates were incubated for 24-24 hours at 37±2°C and the slowly growing isolates were again read after 48 hours incubation. Zone sizes interpreted according to CLSI document criteria for susceptible and resistant.

Quality control: Quality control procedures were performed as per CLSI guidelines using *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 strains, as quality control strains for fluconazole and voriconazole disk diffusion testing.

Results

Oral rinses sample were obtained from 150 patients, 54.7% (n=82) females and 45.3% (n=68) males, most patients were between 20 and 60 years old with over 50% culture positive for yeasts. Female was more colonized by yeast than male and high yeast colonization was also found among patient age from 20 to 40 years old, (Table 1).

| Variable analysis | Total number | Positive culture | % |
|---|---|---|---|
| Oral cavity sample | 150 | 96 | 64 |
| Sex | | | |
| Female | 82 | 55 | 7.1 |
| Male | 68 | 41 | 60.3 |
| Total | 150 | 96 | 64 |
| Age distribution | | | |
| ≤ than 20 | 7 | 5 | 71.4 |
| ≤ than 40 | 55 | 40 | 72.7 |
| ≤ than 60 | 69 | 38 | 55.1 |
| > 60 | 19 | 13 | 68.4 |

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Table 3: Antifungal susceptibility profile of Candida isolates by disk diffusion technique

| Species                  | AMB | FLU25 | FLI100 | POSS | NY10 | CLO50 | ITCS5 | AFY1 | VOR | CAS5 | KCA10 | MCL10 | ECN10 |
|--------------------------|-----|-------|--------|------|------|-------|-------|------|-----|------|-------|-------|-------|
| Candida albicans         | 100 | 26.3  | 26.3   | 23.7 | 100  | 100   | 26.3  | 5.3  | 26.3| 100  | 63.2  | 100   | 100   |
| Candida glabrata         | 100 | 80.8  | 80.8   | 80.8 | 100  | 100   | 76.9  | 7.7  | 76.9| 100  | 80.5  | 100   | 100   |
| Candida dubliniensis     | 100 | 100   | 100    | 100  | 100  | 100   | 100   | 9.1  | 90.9| 100  | 100   | 100   | 100   |
| Candida famata           | 100 | 83.3  | 83.3   | 66.7 | 100  | 100   | 83.3  | 0    | 83.3| 100  | 100   | 100   | 100   |
| Candida guillermondii    | 100 | 100   | 100    | 100  | 100  | 100   | 100   | 50   | 100 | 100  | 100   | 100   | 100   |
| Other yeast              | 100 | 81.8  | 81.8   | 81.7 | 100  | 100   | 81.8  | 18.2 | 81.8| 100  | 100   | 100   | 100   |

Table 2: Type and frequency of yeast species isolated from oral rinse and teeth site specimens

| Oral rinse species | Total no | %     | Site species | Total number | %     |
|--------------------|----------|-------|--------------|--------------|-------|
| Candida albicans   | 40       | 41.7  | Candida albicans | 12           | 36.4  |
| Candida glabrata   | 26       | 27.1  | Candida glabrata | 9            | 27.3  |
| Candida dubliniensis | 11      | 11.5  | Candida dubliniensis | 8           | 24.2  |
| Candida famata     | 6        | 6.3   | Cryptococcus laurenti | 2           | 6.1   |
| Cryptococcus humicola | 4     | 4.2   | Candida famata | 1            | 3     |
| Cryptococcus laurenti | 4      | 4.2   | Cryptococcus humicola | 1           | 3     |
| Candida guillermondii | 2      | 2.1   |              |              |       |
| Trichosporum mucoides | 1      | 1     |              |              |       |
| Saccharomyces cerevisiae | 1 | 1    |              |              |       |
| Rhodotorula minuta | 1        | 1     |              |              |       |
| Total              | 96/150   | Total | 33/91        |              |       |
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Figure 1 Yeast positive culture in patient with various risk factors with reference to positive

DC, dental complaint; PF, predisposing factors; NAD, no apparent disease.

Discussion

The present study to our knowledge is the first study to survey the prevalence of oral Candida species in Tripoli, Libya. The aim of this study was to investigate the carriage rate and background factors underlying carriage of oral Candida species in patient attending outpatient dental clinic in Tripoli district area. The carriage of Candida spp. in the oral cavities was 96 (64%) overall. High oral carriage of yeast either in sex gender or age group was associated in all positive cultures with the presence of at least one dental compliment, in which 127 have at least one dental disease and 81 (63.8%), was positive for yeast. In contrast to patient with no apparent disease (NAD), 23 and only 15 was culture positive for yeast (65.2%), however 15 out 23 at least has one predisposing factor, this may explain high rate yeast carriage in NAD patient. Various study show that high oral carriage of candida in female than male, which may be attributed to the fact that female have more predisposing factors to be colonized and infected than male. Different study showed similar result indicates the importance role of dental compliment particular dental disease gingivitis as well as Periodontitis in candida colonization. The rule of candida species especially candida albicans in oral cavity has been investigated and found to possess nainny virulence factors such as biofilm, adhesion and others to paly important role in colonization and infection. This may explain its high frequent isolation in our study and others. Diabetes and pregnancy are the most important single Predisposing factor for yeast oral carriage in our study, this may also be related the fact that pregnancy and diabetes more prone and susceptible to candida colonization and infection not only oral site but also other such vaginitis in female. Our study also shows that most patient attending dental clinic with dental compliant as well as at least one predisposing factor. This may explain the high rate oral carriage by yeast and importance of both risk factors in oral colonization and infection. Considerable high carriage among our patients with no dental compliant and predisposing factor may be attributed to poor dental hygiene and cleaning.

Isolation of C. glabrata and C. dubliniensis in our study as the most important second pathogen to be isolated from oral cavity was similar to other study. This finding can be related to the fact that both species has been emerged as an important pathogen in the last few years among immunocompromised patient and oral candidiasis either due antifungal resistant as with C. glabrata or virulence factors in C. Dublinsiensis.

All Candida isolates tested were susceptible to amphotericin B, nystatin, caspofungin, clotrimazole, miconazole and econazole. Furthermore, all C. albicans show high resistant to fluconazole, itraconazole, posaconazole and voriconazole. In contrast all other candida species including C. dublinsiensis isolates were susceptible to mostazole. However, C. glabrata isolates showed reduced susceptibility to fluconazole, itraconazole, posaconazole and voriconazole and some of these isolates were also resistant to ketoconazole. All candida species including other yeast were highly resistant to fluocytosine, this due innately resistant or rapid developed resistance. Resistance of C. albicans to fluconazole and other new azole possibly due to cross resistant between these classes of antifungal and have been reported by other studies as observed in our study. This is finding may guide our empiric treatment to shift for old azole in high-risk patients with known predisposing factors from developing serious candida infection particular with C. albicans. Identification of the infecting species such as C. glabrata or C. dubliniensis and other non-candida albicans could also be highly predictive of the likely drug susceptibility to azoles and could be used as a guide to therapy.

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Conflict of interest

Author declares that there is no conflict of interest.

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