Incidence rate of *Candida* species in the oral cavity of middle-aged and elderly subjects

Zaremba ML[1]*, Daniluk T[1], Rożkiewicz D[2], Cylwik-Rokicka D[3], Kierklo A[4], Tokajuk G[5], Dąbrowska E[6], Pawińska M[7], Klimiuk A[7], Stokowska W[7], Abdelrazek S[8]

[1] Department of Microbiology, Medical University of Białystok, Poland
[2] Department of Pediatric Infectious Diseases, Medical University of Białystok, Poland
[3] Department of Prosthodontics, Medical University of Białystok, Poland
[4] Department of Dentistry Propaedeutics, Medical University of Białystok, Poland
[5] Department of Periodontal and Oral Mucosa Diseases, Medical University of Białystok, Poland
[6] Department of Social and Preventive Dentistry, Medical University of Białystok, Poland
[7] Department of Conservative Dentistry, Medical University of Białystok, Poland
[8] Department of Nuclear Medicine, Medical University of Białystok, Poland

Abstract

Purpose: The aim of this study was to determine the incidence rate of oral *Candida* species in middle-aged and elderly subjects.

Material and methods: The study carried out in 103 adults aged 35-92 years, in which 32 (31.1%) used complet or partial acrylic dentures. Mycological tests were performed by using culture (Sabouraud agar) and API 20C AUX (bioMérieux) for identification of the species level. Material for analysis included swabs taken from the palate mucosa and mucosal part of denture surfaces in denture wearers, as well as, from tooth surface and/or dentine carious lesions. The dental caries status of each patients was evaluated using DMF index (WHO 1986 criteria).

Results: Yeasts of *Candida* genus were isolated in 65/103 (63.1%) adults. The incidence rate of *Candida* spp. was higher in adults without dentures (46/71; 64.8%) compared to denture wearers (19/32; 59.4%); however, the differences were not statistically significant ($p=0.59 > p=0.05$). *Candida albicans* were the most frequently isolated species, and with a comparable rate ($p=0.06$), both in adults with and without dentures (17/32; 53.1% and 38/71; 53.5%, respectively). In 3 individuals without dentures, two other species were found apart from *C. albicans*, namely *C. glabrata* (2x) and *C. krusei* (1x). In a total of 11/49 (22.5%) strains belonging to 5 non- *C. albicans* species were detected in adults without dentures, while in denture wearers only 2/19 (10.5%) other species were found (*C. krusei* and *C. oralis*) ($p=0.26 > p=0.05$). Strains of *C. glabrata* species were isolated only from the elderly. No significant differences were noted in the incidence of *Candida* spp. between middle-aged subjects (35-44 years) (35/52; 67.3%) and the elderly (>55 years) (30/51; 58.8%) ($p>0.05$), both in denture wearers and non-denture wearing subjects. However, the frequency of oral *Candida* spp. strains was increased in advanced age subgroup 71-92 years (74.2%) compared with 56-70 years (35.0%) of elderly subjects ($p<0.05$), only in denture wearers (30.0% vs 5.0%) ($p<0.05$). The sex and DMF index distribution of both subject groups had no significant influence on the numbers of *Candida* spp. detected.

Conclusions: Yeasts of the genus *Candida* were isolated at a comparable rate ($p>0.05$) from the oral cavity of adults with and without dentures, as well as in middle-aged (35--44 years) and elderly subjects (56-92 years). However, a significant difference was observed only between elderly subgroups aged 56-70 (35%) and advanced age subgroup 71-92 years (74%).

Key words: adult subjects, denture wearers, oral *Candida albicans*, non-*C. albicans* species, DMF index.

Introduction

Candida species are ubiquitous yeasts and common residents of mucosal surfaces of the human oral cavity, the gastrointestinal and the urogenital tract [1-4]. Essentially all areas of the human gastrointestinal tract can harbor *Candida*. The most commonly isolated species (50 to 70% of yeast isolates) from the human gastrointestinal tract is *Candida albicans*, followed by *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* [3] .

Candida spp. can be present in clinical specimens as a result of environmental contamination, colonization, or actual disease processes. An accurate diagnosis requires proper handling of clinical material. *Candida* spp. that are members of the normal microbiota with high prevalence in the normal population, can invade tissue and cause oral candidosis (candidiasis) or

* CORRESPONDING AUTHOR:
Department of Microbiology
Medical University of Białystok
ul. Mickiewicza 2C, 15-230 Białystok, Poland
e-mail: zaremba@ amb.edu.pl (Maria Lucyna Zaremba)

Received 15.03.2006 Accepted 30.03.2006

Table 1. **Isolation frequency (%) of oral *Candida* spp. in middle-aged and elderly subjects without dentures**

	35-44 years n=41	>55 years n=30	Total n=71
C. albicans	21 (51.2)	14 (46.7)	35 (49.3)
C. albicans + *C. glabrata*		2 (6.7)	2 (2.8)
C. albicans + *C. krusei*	1 (2.4)		1 (1.4)
C. glabrata		2 (6.7)	2 (2.8)
C. krusei		1 (3.3)	1 (1.4)
C. lusitaniae	2 (4.9)		2 (2.8)
C. pelliculosa	1 (2.4)		1 (1.4)
C. pulcherrima	2 (4.9)		2 (2.8)
Candida spp.	27 (65.8)	19 (63.3)	46 (64.8)

Table 2. **Isolation frequency (%) of oral *Candida* spp. in middle-aged and elderly subjects with dentures**

	35-44 years n=11	>55 years n=21	Total n=32
C. albicans	7 (63.6)	10 (47.6)	17 (53.1)
C. krusei		1 (4.8)	1 (3.1)
C. oralis	1 (9.1)		1 (3.1)
Candida spp.	8 (72.7)	11 (52.3)	19 (59.4)

life-threatening disease in patients whose immune defenses have been altered by old age, disease or iatrogenic intervention [1-6]. Mucocutaneous forms of candidiasis are often related to defects in cell-mediated immunity, while systemic spread is generally associated with neutropenia [2,4-7].

As a result of increasing numbers of immunocompromised individuals within the human population, the incidence of *Candida* infections has increased dramatically in the last decade [4-6].

Among species of the genus *Candida*, *C. albicans* is the prevalent causative agent of candidiasis and constitutes the fourth most common nosocomial bloodstream isolate in industrial countries [4,6]. It is generally belived that candidiasis arises from endogenous commensal strains inhabiting the oral cavity, gastrointestinal tract and genitourinary system [3,4,7-9].

Cannon et al. [9] have proposed that *Candida* colonization of oral surfaces, including the denture-fitting surface, can serve as a reservoir for disseminated infections such as aspirate pneumonia and gastrointestinal infections.

The aim of this study was to determine the incidence rate of oral *Candida* species in middle-aged and elderly subjects.

Material and methods

The study carried out in 103 adults, aged 32-92 years, in which 32 (31.1%) used complet or partial acrylic dentures.

Dental epidemiological examinations of the patients from Białystok, and the surroundings carried out in two groups: 1. From dental outpatients clinic 52 patients (35 female and 17 male) aged 35-44 years (middle-aged), 2. Patients from the Geriatric Social Center of Białystok, 51 residence (27 female and 24 male), older than 55 years (56-92 years) (elderly subjects).

History and clinical examinations done using a probe, a mirror, and a WHO periodontometer. Data was recorded on special WHO card (Oral Health Assessment form 1986) used for dental epidemiological assessment.

Dental status evaluated by using of mean value calculated by DMF index, which indicate the severity of caries (D= number of teeth with decay, M= number of missing teeth, F= number of filling teeth).

Mycological tests were performed in all the patients and included culture (Sabouraud agar) and identification to the species level (API 20C AUX; bioMérieux) [4]. The swabs taken from the palate mucosa and mucosal part of denture surfaces in denture wearers, as well as from tooth surface and/or dentine carious were analysed.

The local ethics committee approved this study, and all subjects gave informed consent to the procedures.

The statistical analysis was done using the chi-square test ($p \leq 0.05$).

Results

Yeasts of *Candida* genus were isolated in 65/103 (63.1%) adults. Among them *Candida albicans* species predominate (52/65; 80.0%) ($p=0.0001$). The incidence rate of *Candida* spp. was higher in adults without dentures (46/71; 64.8%) (*Tab. 1*) than with denture (19/32; 59.4%) (*Tab. 2*); however, the differences were not statistically significant ($p=0.59>p=0.05$).

Candida albicans were the most frequently isolated species, and with a comparable rate ($p=0.06$), both in adults with and without dentures (17/32; 53.1% and 38/71; 53.5%, respectively) (*Tab. 1* and *2*). In 3 individuals without dentures, two other species were found apart from *C. albicans*, namely *C. glabrata* (2x) and *C. krusei* (1x) (*Tab. 1*). A total of 11/49 (22.5%) strains belonging to 5 non-*C. albicans* species were detected in adults

***Table 3*. Isolation frequency (%) of oral *Candida* spp. in elderly subjects with and without dentures**

	56-70 years			71-92 years		
	Denture n=8	No-denture n=12	Total n=20	Denture n=13	No-denture n=18	Total n=31
C. albicans	1 (12.5)	3 (25.0)	4 (20.0)	9 (69.2)	11 (61.1)	20 (64.5)
C. albicans + *C.glabrata*		1 (8.3)	1 (5.0)		1 (5.6)	1 (3.2)
C. glabrata		1 (8.3)	1 (5.0)		1 (5.6)	1 (3.2)
C. krusei		1 (8.3)	1 (5.0)	1 (7.7)		1 (3.2)
Candida spp.	1 (12.5)	6 (25.0)	7 (35.0)	10 (76.9)	13 (72.2)	23 (74.2)

without dentures, while only 2/19 (10.5%) other species were found (*C. krusei* and *C. oralis*) in denture wearers (*Tab. 2*) p=0.26 >p=0.05). Strains of *C. glabrata* species were isolated only from the elderly (>55 years) (*Tab. 1*). No significant differences were noted in the incidence of *Candida* spp. between middle-aged subjects (35-44 years) (35/52; 67.3%) and the elderly (>55 years) (30/51; 58.8%) (p>0.05), both in denture wearers (*Tab. 1*) (p=0.826) and non-denture wearing subjects (*Tab. 2*) (p=0.515). However, a significant difference was observed only between elderly subgroups aged 56-70 and 71-92 years old (*Tab. 3*).

The isolation frequency of oral *Candida* spp. strains was more in the advanced age subgroup 71-92 years (23/31; 74.2%) compared with elderly subjects aged 56-70 years (7/20; 35.0%) (p=0.0034); seen only in denture wearers (10/13; 76.9% and 1/8; 12.5%, respectively), and it was statistically significant (p=0.0155). Among elderly patients aged 56-70 years, *Candida* spp. was most often isolated from patients without dentures (6/20; 30.0% vs 1/20; 5%) (p=0.0375) (*Tab. 3*).

The dental caries severity in adult aged 35-44 years detected by using DMF index was very high, about 21.4; and was higher in 35 females (22.9) than in 17 males (18.25). A major influence on the DMF value in this age group was the number of filling teeth, and F component was 9.9 in whole group (11.5 filling teeth in female and only 6.4 in male). In this age group observed more healthy teeth in male (11.6 healthy teeth) than in female (7.3).

The mean value of DMF for the elderly subject (55-92 years) from the second group was 30.5, and was comparable in 24 male (30.7) and 27 female (30.2). This high value was due to numbers of missing teeth (M), with mean value 26.4 teeth (27.1 in male and 25.6 in female). In this group we observed a few number of filling teeth (mean F=0.2; in female =0.1, in male 0.2), and very few number of teeth present in oral cavity (mean 5.7 teeth, where only 1.5 was healthy). More teeth were in male (mean =6.3) than female (mean =5.1), however, more healthy teeth were seen in female (mean =1.7) compared to male.

The mucous membrane changes of oral cavity were seen in 41(80.4%) elderly subjects (18 female and 23 male). Xerostomia more frequently detected in female (44.4%), while tongue disorders in male (60.9%).

The sex and DMF index distribution of both age groups 35-44 and 56-92 years had no significant influence on the numbers of *Candida* spp. detected.

Discussion

It has generally been assumed that old age represent a predisposing condition for increased candidal colonization. Lockhart et al. [10] demonstrated that frequency and intensity of carriage of candidal colonization increased as a function of age, independent of denture use. However, Ikebe et al. [11] showed that candidal activity was not significantly associated with age or gender in the relatively healthy people. The activity of *Candida* species in the oral cavity was associated with the wearing of removable dentures and stimulated salivary flow, independent of age or gender even in the relatively healthy elderly (mean age of 66.7±4.3 – s.d. – years) [11].

Commensal existence of intraoral *Candida* species varies from 20 to 50% in a healthy edentulous population [12,13] and up to 75% in a population wearing dentures [13,14].

Our previous studies have shown a high incidence of isolation of *Candida* species from oral cavities of healthy middle-aged (about 40 years) denture wearers (75%) and healthy people with their own dentition (41%) [15].

According to our results presented here, the prevalence rate of *Candida* spp. in the oral cavity of adults with (59.4%) and without dentures (64.8%) (p>0.05), as well as in middle-aged (35-44 years) and elderly subjects (56-92 years) was high (67% vs 59%) with comparable rate (p>0.05). However, the frequency of oral *Candida* spp. strains was increased in advanced age subgroup 71-92 years (74.2%) compared with 56-70 years (35.0%) of elderly subjects (p<0.05), only seen in denture wearers (30.0% vs 5.0%) (p<0.05).

Belazi et al. [16] also did not reveal any association between age and *Candida* growth in any of the study groups such as the diabetic patients and the group of healthy subjects with a mean age of 54±7 years (range 40-80 years). Although the results showed an equal prevalence of candidal growth among healthy and diabetic adults wearing dentures. For older age (>60 years old) when combined and presence of dentures, a statistically significantly greater proportion of subjects with diabetes mellitus suffered from candidiasis [16].

We speculate, similar to Belazi et al. [16], that the oral carriage of *Candida* spp. cannot be directly associated with either age or presence of dentures. Certain systemic conditions (e.g. diabetes mellitus), defects in the immune system [4,5,7,10], and/or some medications (e.g. antibiotics, corticosteroids) may predispose the transformation of a benign colonization, such as *Candida* species, into opportunistic pathogens [19]. Denture use

and hyposalivation are common not only in the frail elderly, but also in the comparatively healthy elderly who live independently [11,20]. The Japanese national survey of dental diseases by the Ministry of Health and Welfare collected data from every member of all households in 300 municipalities that had been randomly sampled in every prefecture [11]. The survey found that the number of residual teeth gradually declined after age 50. Consequently, 50% of people between 65 and 74 years and 70% of those between 75 and 84 used removable dentures.

Tokajuk et al. [21] evaluated prevalence of toothlessness in 591 subjects of geriatric population of the north-eastern of Poland and found that 37.0% of them were edentulous. The study supervised by WHO, indicate that there is a need for prosthetic treatment in elderly subjects, especially in older age groups (65-74 years and ≥75 years old) and who lived in institutions. Stokowska et al. [22] examined the middle-aged adults (35-44 years) living in Białystok during epidemiological studies carried out in 1987 and 1995. High mean DMF values were found in both 1987 (18.14) and 1995 (17.24). These values were higher than for all Polish population (18.6 and 16.5, respectively).

Our study showed that DMF indices both in middle-aged adults (35-44 years) (21.4) and in elderly subjects (55-92 years) (30.5) were very high. However, DMF index and sex distribution of both subject groups had no significant influence on the number of *Candida* species detected.

References

1. Odds FC. *Candida* and candidosis. 2nd ed., Balliere Tindall, London, 1988.
2. Hazen KC. New and emerging yeast pathogens. Clin Microbiol Rev, 1995; 8: 462-78.
3. Hazen KC, Howell SA. *Candida*, *Cryptococcus*, and other yeasts of medical importance. In: Manual of clinical microbiology. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. 8th ed., Washington, D.C., American Society for Microbiology Press, 2003; 2: 1693-711.
4. Zaremba ML, Borowski J. Mikrobiologia lekarska. wyd. 3, Warszawa, Wydawnictwo Lekarskie PZWL, 2003.
5. Coleman DC, Bennett DE, Sullivan D, Gallagher PJ, Henman MC, Stanley DB. Oral *Candida* in HIV infection and AIDS: new perspective/new approaches. Crit Rev Microbiol, 1993; 19: 61-82.
6. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. Clin Microbiol Rev, 1997; 10: 444-65.
7. Jacob LS, Flaitz CM, Nichols CM, Hicks MJ. Role of dental caries lesions in the pathogenesis of oral candidiasis in HIV infection. J Am Dent Assoc, 1998; 129: 187-94.
8. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol, 1985; 152: 924-35.
9. Cannon RD, Holmes AR, Mason AB, Monk BC. Oral *Candida*: clearance colonization, or candidiasis? J Dent Res, 1995; 74: 1152-61.
10. Lockhart SR, Joly S, Vargas K, Swails-Wenger J, Enger L, Soll DR. Natural defenses against *Candida* colonization breakdown in the oral cavities of the elderly. J Dent Res, 1999; 78: 857-68.
11. Ikebe K, Morii K, Matsuda K, Hata K, Nokubi T. Association of candidal activity with denture use and salivary flow in symptom – free adults over 60 years. J Oral Rehabil, 2006; 33: 36-42.
12. Liu X, Liu H, Guo Z, Luan W. Association of asymptomatic oral candidal carriage, oral candidiasis and $CD4^+$ lymphocyte count in HIV-positive patients in China. Oral Dis, 2006; 12: 41-4.
13. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials *in vivo* and *in vitro*. Crit Rev Oral Biol Med, 1999; 10: 99-116.
14. Spiechowicz E. Stomatopatie protetyczne. PZWL, Warszawa, 1993.
15. Pietruski JK, Sacha P, Zaremba M, Gołębiewska M, Stokowska W. Yeast infection in denture stomatitis patients. Part I. Fungal flora – assessment. Prot Stom, 1997; 47: 197-202.
16. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, Danülidou N, Karamitos D. Candidal overgrowth in diabetic patients: potential predisposing. Mycoses, 2005; 48: 192-6.
17. Pietruski JK, Kaczmarski W, Jabłońska E, Zaremba M, Stokowska W, Gołębiewska M. Yeast infection in denture stomatitis patients. Part II. Immunoglobulins. Prot Stom, 1997; 47: 288-92.
18. Pietruski JK, Pietruska MD, Jabłońska E, Sacha P, Zaremba ML, Stokowska W. Interleukin 6, tumor necrosis factor alpha and their soluble receptors in the blood serum of patients with denture stomatitis and fungal infection. Arch Immunol Ther Exp, 2000; 48: 101-5.
19. Webb BC, Thomas CJ, Willcox MD, Harly DW, Knox KW. *Candida*-associated denture stomatitis. Aetiology and management: a review. Part 2. Oral disease caused by *Candida* species. Aust Dent J, 1998; 43: 160-6.
20. Bergdahl M. Salivary flow and oral complaints in adults dental patients. Community Dent Oral Epidemiol, 2000; 28: 59-66.
21. Tokajuk G, Stokowska W, Miksza-Żyłkiewicz R. Prevalence of toothlessness in subjects over 55 years living in the north-eastern region of Poland. Gerontologia Polska, 2003; 2: 27-30.
22. Stokowska W, Kierklo A, Marczuk–Kolada G, Kierklo A, Miksza-Żyłkiewicz R, Milewska R, Łuczaj-Cepowicz E. The caries intensity in adult population living in the Białystok district. Czas Stomat, 1999; 52: 523-7.