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RESEARCH ARTICLE

PREVALENCE AND ASSOCIATED FACTORS OF ORAL NON-*CANDIDA ALBICANS CANDIDA* CARRIAGE IN DENTURE WEARERS IN SANA'A CITY-YEMEN

Khaled Abdulsalam Al-Haddad[1], Omar Ahmed Esma'il Al-dossary[2], Hassan A. Al-Shamahy[3]

[1]*Department of Orthodontic Pediatric Dentistry, Faculty of Dentistry, Sana'a University, Sana'a, Yemen.*
[2]*Medical Microbiology Clinical Immunology, Faculty of Medicine Health Sciences, Sana'a University, Republic of Yemen.*
[3]*Faculty of Medicine Heath Sciences, Sana'a University, P.O. Box 775 Sana'a, Yemen.*

ABSTRACT

Objective: The objective of this study was to contrast the prevalence and species of colonization of Non-*Candida albicans* (NCAC) in the oral cavity of denture wearers and non-denture wearers; also asses associated risk factors of their colonization.

Methods: A total of 208 subjects were studied: 104 denture wearers and 104 non-denture wearers, matched by age, sex, comprised the experimental control groups, respectively. Each subject was instructed to perform oral rinsing using a phosphate-buffered saline solution, which was expectorated processed for the recovery of *Candida* species on on Sabouraud dextrose agar. Isolates were identified by culturing on chromogenic *Candida* agar noting species-specific colony characteristics.

Results: There was a significant oral carriage rate of NCAC among denture wearers (5.83% versus 11.1% in controls) with associated risk factor (5.4) (p<0.001). The most common isolated NCAC were *C. Krusi C. tropicalis* with significant OR (5.5, 4.7 respectively). When co-infections were considered, there was highly significant association of *C. albicans* + *C. krusi* oral colonization in cases (OR=4.56, p<0.001). There was a significant oral carriage rate of *NCAC* among male denture wearers (36.9%, OR=6.6, p<0.001). In addition, there was a significant rate of NCAC colonization with complete denture (rate= 50%, OR=2.4, P= 0.02). While no significant increase associated with colonization of NCAC with partial, acrylic /or chrome cobalt denture.

Conclusion: Based on the results of this study ability of NCAC were greater in denture wearers than non-denture wearers, also greater risk of NCAC were found with males, older ages, complete denture.

Keywords: Carriage, denture, non-*Candida albicans andida* (NCAC), prevalence.

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Address for Correspondence:
Prof. Hassan A. Al-Shamahy, Faculty of Medicine Heath Sciences, Sana'a University, P.O. Box 775 Sana'a, Yemen. Tel: +967-1-239551, E-mail: *shmahe@yemen.net.ye*

INTRODUCTION

The preponderance oral yeast infections are caused by members of the genus *Candida*. *Candidiasis* is an opportunistic infection due to pathological changes in the surface of oral mucosal cavity[1,2]. *Candidiasis* patients may show various symptoms including painful sensation, burning, swallowing difficulty change of taste, but most frequently are asymptomatic[3]. The infection is generally treated with antifungal drugs, but in immune-compromised patients such as patients treated in intensive care units, cancer patients receiving radiation or chemotherapy, organ transplant patients HIV-positive patients' return of infections may be problem. In the last twenty years, some *Candida species*, including *C. glabrate*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, have been isolated with increasing frequency from cases of *Candidiasis*[4,5,6]. It is known that each species differs in the production of recognized virulence factors sensitivity to antifungal agents. Isolation identification the *Candida* spp. is useful in choosing correct treatment, as some species may be resistant to certain groups of antifungal drugs[7-9]. Infection caused by *NCAC*, such as *C. glabrate*, *C. tropicalis*, *C. krusei*, have been found to be less response to fluconazole[10]. Moreover, there are reports of numerous cases describing infection colonization of immunocompromised patients on long-term treatments of oral antifungal agents, drug resistant *C. glabrate* C. *krusei* have been recovered from them[11].

As well as, host defenses have been reported to be less effective in patients infected by *C. glabrata* than *C. albicans*[11]. The newer triazoles, including posaconazole voriconazole; echinocandins, caspofungin, micafungin, anidulafungin are antifungal drugs that show strong activity against *Candida* spp. On the other hand, echinocandins, appears to be less potent against some species, such as C. *guilliermondii C. parapsilosis*[8]. As well, *C. dubliniensis* is very similar to *C. albicans* has been reported to have low susceptibility to azole drugs[9,12].

The wearing of dentures is associated with overgrowth of *Candida* species, leading to denture stomatitis. Studies to identify *Candida* species in patients with denture stomatitis have produced contradictory results[8]. A number of studies assertion that a single species was responsible for the infections[13] while others isolated accused multiple species of *Candida*[14]. The objective of this study was to compare the prevalence and species colonization due to NCAC in the oral cavity of denture wearers' non-denture wearers' analysis associated risk factors of their colonization.

METHODS

Subject selection

A total of two hundreds eight persons, were included in this study, 104 of them were denture wearer patients (cases group) while the other 104 adults with natural teeth (controls group), whom been selected from Al-Thawrah hospital, Al-Gumhory hospital Dental centers in Sana'a city, Yemen. The duration of the study was six months period, started in August 2017 ended in February 2018. Inclusion criteria for subject selection were healthy individuals with no clinical signs of *Candida* infection no systemic disease. In addition, individuals who smoked, currently taking antifungal, steroids, antibiotics, or immunosuppressive drugs in the past 6 months were excluded.

Collection identification of samples

Salivary samples were collected using the oral rinse technique[15]. In brief, each subject was asked to rinse the mouth for 60 seconds with 10 ml of sterile phosphate-buffered saline (PBS; 0.01 M phosphate-buffered saline solution, pH 7.2) expectorate the wash into a 15 ml sterile container[16]. Individuals who had removable dentures were asked to take out the denture prior to samples collection. The samples were immediately transported on ice to the microbiology laboratory. Each oral rinse was centrifuged at 3500 rpm for 10 minutes, and then the supernatant was discarded. The pellet was re-suspended in 1ml sterile PBS. Total 100 µl of the concentrated oral rinse was inoculated onto Sabouraudus dextrose agar incubated at 37ºC for 48 hours. The lasting samples were stored at -20ºC. If *Candida* colonies appeared on the Sabouraud dextrose agar, then chromogenic *Candida* agar was inoculated using 100 µl of the oral rinse supernatant incubated for 48 hours for colonies study. *Candida* species were identified by the color of the colonies using the color reference guide supplied by the manufacturer. When color identification was unclear, fermentation assay of sucrose, maltose, glucose, lactose galactose was done. The *Candida* species were also identified by the ability to produce chlamydo-spores on glutinous rice agar[17].

Data analysis

Data were statistically analyzed using the EPI-Info program version 6.The difference in distribution of the *Candida species* between groups was based on comparison of frequency distributions by a chi-square test. A p value < 0.05 was considered significant.

Ethical approval

We obtained written consent from all cases. Assent was taken from participants before collecting the specimens. The study proposal was evaluated and approved by the Ethics Committee of Faculty of Medicine Health Sciences, Sana'a University.

Table 1: The yeast distribution in different sexes of the denture wearer (cases) non denture wearer (controls) groups of the study populations.

Organisms	Cases (No.=104)		Controls (No.=104)		OR	CI	χ^2	*P*
	No.	%	No.	%				
Total Non C. albicans Candida	42	38.5	12	11.5	5	2.4-10.2	35	<0.001
C. krusi	32	30.7	8	7.6	5.5	2.4-12.7	18.8	<0.001
C. tropicalis	16	15.4	4	3.8	4.7	1.5-14.6	8.4	0.003
C. glabrata	2	1.9	2	1.9	1.03	0.14-7.5	0.96	0.6
C. albicans + C. krusi	22	21.1	6	5.8	4.56	1.7-11.7	22	<0.001
C. albicans + C. tropicalis	8	7.7	4	3.8	2.16	0.6-7.4	1.9	0.16
C. albicans + C. krusi + C. tropicalis	6	5.7	0	0.0	undefined		6.5	0.012

OR- Odds ratio = Relative risk, 95%CI- 95% Confidence intervals, χ^2 Chi-square = 3.9 or more is significant, *p*v- Probability value = 0.05

RESULTS

There was a significant oral carriage rate of NCAC among denture wearers (cases) equaled to 38.5% comparing with only 11.1% among non-denture wearers (controls) with OR of association equaled to 5.4 times (p<0.001). There was a significant oral carriage rate of *C. Krusei* among cases (30.7%) comparing with only 7.4% in controls with 5.5 association (p<0.001). A significant oral carriage rate of *C. tropicalis* among cases (15.4 %,) comparing with only 3.7% in controls with 4.7 times of association (P=0.003), while no significant association with *C. glabrata*. When co-infections were considered, there was highly significant association of *C. albicans + C. krusi* oral colonization in cases (OR=4.56, p<0.001),

but no significant association of *C. albicans* + *C. tropicalis* infection in the denture wearer (Table 1). There was a significant oral carriage rate of *NCAC* species among male denture wearers (36.9%) with significant association (OR=6.6, $p<0.001$). While in the females group, there was only a slightly difference between oral carriage rate of NCAC among female denture wearers comparing with non-denture wearer females. There was a highly significant association between older age groups (>45 years) of cases and contract of oral NCAC colonization with OR ranged from 1.4 to 5.4 times (Table 2). When we considered type of dental prosthesis, there was a significant rate increase with complete denture. The rate was 50%, with a highly significant association between complete denture and contract of oral NCAC colonization (OR=2.4, $p=0.02$). While no significant increase associated with colonization of NCAC with partial, acrylic /or chrome cobalt denture (Table 3).

Table 2: The carriage rate of NCAC in cases controls associated OR for different sex's age groups.

Factors		Cases (n=104)		Controls (n=104)		OR	CI	χ^2	*P*
		No.	%	No.	%				
Male		36/92	39.1	8/92	8.7	6.6	2.7 -16.5	23	<0.001
Female		6/12	50	4/12	33.3	1.6	0.4-6.9	0.5	0.47
Age group	< 45 years	2/10	20	2/10	20	1.4	0.1- 10	0.0	0.97
	45-54 years	8/16	50	2/16	12.5	4.4	1.0 -30	4	0.04
	55 – 64 years	16/32	50	4/32	12.5	4.7	1.4-17.4	8.5	0.003
	≥ 65 years	18/46	39.1	4/46	8.7	5.4	1.6-19.8	10.5	0.001
Total		42/104	40.4	12/104	11.5	5.4	2.5-11.8	23.9	<0.001

OR- Odds ratio = Relative risk, 95%CI- 95% Confidence intervals, χ^2 Chi-square = 3.9 or more is significant, *pv*- Probability value = 0.05

Table 3: The type of dental prosthesis factors that associated with colonization of NCAC among denture wearer patients.

Factors	Positive NCAC (n=42)		OR	CI	χ^2	*P*
	No.	%				
Type of dental prosthesis						
Complete n=46	24	52.2	2.4	1.0.- 5.8	4.7	0.02
Partial n=58	18	31	0.4	0.16- 0.9	5.4	0.02
Acrylic n=74	30	40.5	1.02	0.4-2.6	0.00	0.97
Chrome cobalt n=38	12	31.6	0.73	0.3-1.8	0.54	0.46

OR- Odds ratio = Relative risk, 95%CI- 95% Confidence intervals, χ^2 Chi-square = 3.9 or more is significant, *pv*- Probability value = 0.05

DISCUSSION

NCAC strains, however, are isolated in growing numbers in medically compromised patients. These strains might cause systemic infections are frequently resistant to commonly used antifungal agents such as fluconazole[18,19]. In the present study, there was a significant oral carriage rate of NCAC among denture wearers (38.5%) comparing with only 11.1% among non-denture wearers. In addition, there was a highly significant association between denture wear and contract of oral NCAC with OR equaled to 5.4 times ($p<0.001$). When single species of NCAC was considered, there was a significant oral carriage rate of *C. Krusei* among cases (30.7%) comparing with only 7.4% in controls with 5.5 association ($p<0.001$) followed by *C. tropicalis* (15.4%) comparing with only 3.7% in controls with 4.7 times of association ($p=0.003$), while no significant association with *C. glabrata* and absent of *C. parapsilosis* (Table 1). Current results were different from that reported elsewhere, in which *C. tropicalis* was the most common NCAC, followed by *C. glabrata*, while *C. parapsilosis* were rare isolated in healthy oral colonization or as a cause of illnesses in patients[19].

When co-infections were considered, there was highly significant association of *C. albicans* + *C. krusi* oral colonization in cases (OR=4.56, $p<0.001$), but no significant association of *C. albicans* + *C. tropicalis infection* in the denture wearer (Table 1). The carriage rates of single multiple *Candida* species were reported to be significantly higher in denture wearers[20]. As it is known, NCAC may be capable of metabolizing ethanol to carcinogenic acetaldehyde can thus progress oral upper gastrointestinal tract cancer. So, 38.5% of current studied individuals having dentures under possible risk of oral upper gastrointestinal tract cancer. Consequently, more focus should be placed on diagnosis treatment of oral *Candida* infections, also on other *Candida* species than *C. albicans* as it has been recommended[19,20].

The highly significant association between denture wear a contract of oral NCAC with the high risk (OR equaled to 5.4 times) can be explained by the fact that one of the most important virulence factors of NCAC is its ability to form biofilms, which has an important clinical consequence, as it confers resistance to antifungal therapy capacity for yeast cells within the biofilms to resist host immune defenses[23-26]. A second explanation might be due to that changes in the oral environment affected by tooth loss or denture wearing

can cause changes in oral microflora[20]. Also in this study, the data supported the rejection of the null hypothesis, which states that there would be no difference between male and female denture wearers in terms of the colonization by NCAC of the inner surfaces of dentures and the surrounding attachments. While there was a significant association between denture wearing in male group a contract of oral carriage of NCAC with OR equaled to 6.6 times, (CI=2.7 -16.5, p<0.001). Current result was different from that reported by Thein[27] in *which* similar colonization rate was observed in both adult sexes. Moreover, there was a trend of rising of oral NCAC colonization with increasing age in cases and controls (Table 1), in which the highest rate of NCAC was in the older age groups. Current study's result, also, was similar to that reported by Chopde *et al.*,[28] in which extremes of age is more susceptible to oral NCAC colonization. This result can be explained by that the elderly generally suffered from systemic illnesses, changes in nourishment and their salivary characteristics[28,29]. Advancing age was also a risk factor for denture stomatitis in the elderly, because cell-mediated immunity, which provides protection against *Candidal* infection, declines with age. According to Dar-odeh Shehabi, some oral environmental factors, such as un-stimulated salivary flow rate age of subjects, were associated with higher numbers of microbes in the saliva of complete denture wearers[30]. Those authors suggested that a reduction in the salivary flow rate with aging induced an increase in concentration of microbes in saliva[31].

CONCLUSION

Based on the results of this study ability of NCAC were greater in denture wearers than non-denture wearers, also greater risk of NCAC were found with males, older ages, complete denture. In addition, current results are important for the development of strategies for eliminate these indicators of risk significantly reduce N*CAC* colonization oral *Candida* infections in denture wearers.

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AUTHOR'S CONTRIBUTION

Al-Haddad KA: writing original draft, conceptualization. **Al-dossary OAE:** methodology, investigation. **Al-Shamahy HA:** writing, review, and editing, supervision, resources.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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