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

Khaled Abdulsalam Al-Haddad1, Omar Ahmed Esma’il Al-dossary2, Hassan A. Al-Shamahy3

1Department of Orthodontic Pediatric Dentistry, Faculty of Dentistry, Sana’a University, Sana’a, Yemen
2Medical Microbiology Clinical Immunology, Faculty of Medicine Health Sciences, Sana’a University, Republic of Yemen.
3Faculty of Medicine Health 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 assess 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 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 (5.8% versus 11.1% in controls) with associated risk factors (5.4) (PV<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. kruisi oral colonization in cases (OR=4.56, PV<0.001). There was a significant oral carriage rate of NCAC among male denture wearers (36.9%, OR=6.6, PV<0.001). In addition, there was a significant rate of NCAC colonization with complete denture (rate= 50%, OR=2.4, PV= 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 andia (NCAC), prevalence.

INTRODUCTION

The preponderance oral yeast infections are caused by members of the genus Candida. Candida. Candidiasis is an opportunistic infection due to pathological changes in the surface of oral mucosal cavity1,2. Candidiasis patients may show various symptoms including painful sensation, burning, swallowing difficulty change of taste, but most frequently are asymptomatic3. The infection is generally treated with anti fungal 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. glabrata, C. tropicalis, C. krusei, C. parapsilosis, have been isolated with increasing frequency from cases of Candidiasis4,5,6. 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 drugs7,8. Infection caused by NCAC, such as C. glabrata, C. tropicalis, C. krusei, have been found to be less response to fluconazole9,10. Moreover, there are reports of numerous cases describing infection colonization of immune-compromised patients on long-term treatments of oral antifungal agents, drug resistant
C. glabrata C. krusei have been recovered from them. As well as, host defenses have been reported to be less effective in patients infected by C. glabrata than C. albicans. 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. As well, C. dubliniensis is very similar to C. albicans has been reported to have low susceptibility to azole drugs.

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. A number of studies assertion that a single species was responsible for the infections while others isolated accused multiple species of Candida. 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 romly 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. 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. 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. One hundred µl of the concentrated oral rinse was inoculated onto Sabouraud dextrose agar incubated at 37°C for 48 hours. The lasting samples were stored at -20°C. 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.

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

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Cases (No.=104)</th>
<th>Controls (No.=104)</th>
<th>OR</th>
<th>CI</th>
<th>χ²</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Non C. albicans Candida</td>
<td>42 38.5 12 11.5 5 2.4-10.2 35 &lt;0.001</td>
<td>32 30.7 8 7.6 5.5 2.4-12.7 18.8 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>16 15.4 4 3.8 4.7 1.5-14.6 8.4 0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>2 1.9 2 1.9 1.03 0.14-7.5 0.96 0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>22 21.1 6 5.8 4.56 1.7-11.7 22 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans + C. krusei</td>
<td>8 7.7 4 3.8 2.16 0.6-7.4 1.9 0.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans + C. tropicalis</td>
<td>6 5.7 0 0.0 undefined 6.5 0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR- Odds ratio = Relative risk, 95%CI- 95% Confidence intervals, χ² Chi-square = 3.9 or more is significant, PV- Probability value = 0.05 or less is significant.

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 (PV<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 (PV<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 (PV=0.003), while no significant association with C. glabrata. When co-infections were considered, there was highly significant association of C. albicans + C. krusei oral colonization in cases (OR=4.56, PV<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, PV˂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, PV=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**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Cases (n=104)</th>
<th>Controls (n=104)</th>
<th>OR</th>
<th>CI</th>
<th>χ²</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36/92</td>
<td>39.1</td>
<td>8/92</td>
<td>8.7</td>
<td>6.6</td>
<td>2.7-16.5</td>
</tr>
<tr>
<td>Female</td>
<td>6/12</td>
<td>50</td>
<td>4/12</td>
<td>33.3</td>
<td>1.6</td>
<td>0.4-6.9</td>
</tr>
<tr>
<td>&lt; 45 years</td>
<td>2/10</td>
<td>20</td>
<td>2/10</td>
<td>20</td>
<td>1.4</td>
<td>0.1-10</td>
</tr>
<tr>
<td>45-54 years</td>
<td>8/16</td>
<td>50</td>
<td>2/16</td>
<td>12.5</td>
<td>4.4</td>
<td>1.0-30</td>
</tr>
<tr>
<td>55 – 64 years</td>
<td>16/32</td>
<td>50</td>
<td>4/32</td>
<td>12.5</td>
<td>4.7</td>
<td>1.4-17.4</td>
</tr>
<tr>
<td>≥ 65 years</td>
<td>18/46</td>
<td>39.1</td>
<td>4/46</td>
<td>8.7</td>
<td>5.4</td>
<td>1.6-19.8</td>
</tr>
<tr>
<td>Total</td>
<td>42/104</td>
<td>40.4</td>
<td>12/104</td>
<td>11.5</td>
<td>5.4</td>
<td>2.5-11.8</td>
</tr>
</tbody>
</table>

OR- Odds ratio = Relative risk, 95%CI- 95% Confidence intervals, χ² Chi-square = 3.9 or more is significant, pv- Probability value = 0.05 or less is significant

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

<table>
<thead>
<tr>
<th>Factors</th>
<th>Positive NCAC (n=42 )</th>
<th>OR</th>
<th>CI</th>
<th>χ²</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of dental prosthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete n=46</td>
<td>24</td>
<td>52.2</td>
<td>2.4</td>
<td>1.0- 5.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Partial n=58</td>
<td>18</td>
<td>31</td>
<td>0.4</td>
<td>0.16- 0.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Acrylic n=74</td>
<td>30</td>
<td>40.5</td>
<td>1.02</td>
<td>0.4- 2.6</td>
<td>0.00</td>
</tr>
<tr>
<td>Chrome cobalt n=38</td>
<td>12</td>
<td>31.6</td>
<td>0.73</td>
<td>0.3-1.8</td>
<td>0.54</td>
</tr>
</tbody>
</table>

OR- Odds ratio = Relative risk, 95%CI- 95% Confidence intervals, χ² Chi-square = 3.9 or more is significant, pv- Probability value = 0.05 or less is significant

**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. 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 (PV<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 (PV<0.001) followed by C. tropicalis (15.4%) comparing with only 3.7% in controls with 4.7 times of association (PV=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.

When co-infections were considered, there was highly significant association of C. albicans + C. krusi oral colonization in cases (OR=4.56, PV<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 wearer. As it is known, NCAC may be capable of metabolizing ethanol to carcinogenic acetaldehyde and 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.

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. 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. 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, PV<0.001). Current result was different...
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 NCAC colonization oral Candida infections in denture wearers.

ACKNOWLEDGMENTS

Authors acknowledge the financial support of Sana’a University, Yemen.

AUTHOR’S CONTRIBUTION

The manuscript was carried out, written, and approved in collaboration with all authors.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES

https://doi.org/10.1590/s1678-77522008000200002

https://doi.org/10.1177/1544073706001900124

https://doi.org/10.1590/S0036-46652006000500004

https://doi.org/10.1128/JCM.41.7.2961-2967.2003

https://doi.org/10.1016/j.archoralbio.2007.01.009

https://doi.org/10.1177/154407370601900124

https://doi.org/10.1186/1471-2180-14-61

https://doi.org/10.1046/j.1439-0507.2003.00871.x

https://doi.org/10.1590/s1517-74912000000200005