Original Article

Study of the oral carriage of Candida sp. in dental students and staff—Identification of Candida sp. and background survey

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ABSTRACT

Purpose: The purpose of this study is to investigate the relationship between oral candidal carriage in able-bodied persons (dental students and staff) and health condition using an epidemiological method with questionnaire, and use the results for an educational campaign for the promotion of health.

Methods: The candidal carriage was examined by culture method using swabs from tongue surfaces. The identification of Candida spp. of the culture positive specimens was performed by a multiplex polymerase chain reaction method. Questionnaire items included age, gender, body mass index, pedometer use, oral conditions, regularity of hospital visits, medication, dental visits, oral care, exercise habits, alcohol habits, and smoking habits.

Results: A total 482 participants were surveyed over a period of two years, males: 269 (mean, 36 years); females: 213 (33 years). Oral candidal carriage was 18.3%. Candida albicans accounted for 80.7% of isolated Candida spp. After analysis using a stepwise method, three items (age, smoking habits, and exercise habits) were selected as the variables in the model. The adjusted odds ratios (95% confidence intervals) for the three model variables were 1.84 (1.10–3.08) for exercise habits, 1.93 (1.00–3.69) for smoking, and 0.96 (0.94–0.98) for age. Logistic regression analysis suggested an association between candidal carriage, exercise habits, and smoking.

Conclusions: Because lack of exercise, as well as smoking are well-known to be detrimental factors to the maintenance of good health, candidal carriers do not appear to be in a condition of good health. In other words, candidal carriage may be a health warning.

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1. Introduction

The fungus Candida, which exists as a benign member of the normal mucosal flora, commonly causes a mucosal disease with substantial morbidity, and in vulnerable patients may cause life-threatening bloodstream infections as an opportunistic pathogen [1–3]. The pathogenicity of Candida albicans is the strongest among the Candida genus members [4]. A striking biological feature of this species is its ability to grow in yeast, pseudo-hyphal, and hyphal forms. The hyphal form has an important role in causing disease by invading epithelial cells and causing tissue damage [5]. In addition, the biofilm formation ability of C. albicans is known to be deeply involved in its pathogenicity. The production of quorum-sensing molecules by C. albicans, such as farnesol [6] and tyrosol [7], is actively studied to control the initial colonization of C. albicans in the oral cavity [8]. The isolation rates of Candida spp. in the oral cavity vary widely from 25 to 75%, depending on the population sampled and the sensitivity of the sampling method [3, 9–12]. Although Candida spp. are detected in relatively high rates in the oral cavity, it is considered to be rare for a healthy person to develop candidiasis [2]. However, once abnormal fungal growth has begun, it will become pathogenic. For example, when taking certain medications, especially antibiotics, fungus in the oral cavity is likely to grow in immune-compromised patients [3]. It is known that the occurrence of oral candidiasis in a human immunodeficiency virus infected patients is an indicator of the subsequent progress of full-blown acquired immune deficiency syndrome [13, 14]. In this context, it should be noted that oral candidiasis is generally regarded as a sign of impaired local or systemic defense mechanisms [15]. However, little is known about the relationship between health and oral candidal carriage in healthy individuals.

Because Candida spp. are thought to have a commensal relationship with normal bacterial flora, it appears that there has been a tendency to discount them as simple commensals, taking it for granted their frequent presence in the oral cavities of healthy persons. Accordingly, few attempts have heretofore been made to investigate the relationship between candidal carriage and health conditions in healthy subjects.
In this work, we planned a study to investigate the relationship between oral candidal carriage and the health conditions of individuals using epidemiological methods, based on the suspicion that the existence of minor host defense system failures, which are undetectable by existing diagnostic and/or laboratory examinations, might be progressing in candidal carriers. In other words, it may be possible to guess the health condition of a subject by testing the existence or nonexistence of candidal carriage in an outpatient clinic. Based on these results, we believe that we may contribute to health promotion.

2. Materials and methods

2.1. Subjects

Healthy volunteers among the students and staff at The Niigata Dental University Niigata (NDUN) participated in this study for 2 consecutive years. Although these volunteers were selected according to the definition of health by the World Health Organization, patients with well-controlled conditions, such as those with mild hypertension who lived without hindrance, were counted as healthy persons.

Included students were selected 2nd grade students in their first year, and selected 3rd grade students in their 2nd years. With regard to the student group, the current candida carriage ratio was compared with that from our previous study, undertaken 15 years previously [11].

2.2. Specimen collection/fungal culture

Specimen collection and culture were carried out according to our previous study [11].

Briefly, specimens on the tongue were collected by using a cotton swab, and then cultured for 48 h in Sabouraud agar plates containing antibiotics (Eiken Kagaku Co., Ltd., Tokyo, Japan). The tongue was selected as the location for sampling, because that is the location where Candida spp. are most often found, particularly in the posterior dorsum area in the circumvallate papillae. After collecting the living colonies grown on agar, a colony suspension was prepared to provide DNA template for multiplex polymerase chain (PCR) reaction. Therefore, we identified the Candida cells in the oral cavity which possessed the ability to grow on Sabouraud agar plates. If necessary, CHROMagar Candida (Kanto Chemical Co., Inc., Tokyo, Japan) was secondarily used to isolate Candida spp.

2.3. Identification of Candida spp.

The resulting Candida species were identified via multiplex PCR.

2.3.1. Primer design

We designed the primer set for multiplex PCR to enable the discrimination of 7 specific species (C. albicans, C. dubliniensis, C. glabrata, C. guilliermondii, C. krusei, C. parapsilosis, and C. tropicalis) from 5 other Candida spp. (Candida famata, C. kefyr, C. lusitaniae, C. pelliculosa, and C. utilis) (Fig. 1) and Saccharomyces cerevisiae, based on the nucleotide sequences of each strain, registered in GenBank (Table 1). The sequence of each band amplified by multiplex PCR, using the above primer set completely corresponded to the specific sequence for each species registered in GenBank. The validity of multiplex PCR was checked by the local alignment of specific band against the corresponding gene sequence.

2.4. Direct multiplex PCR method

Fresh cells from Candida spp. were suspended in 50 μl of 25 mM NaOH. After freezing and thawing, the suspension was

<table>
<thead>
<tr>
<th>Identification of Candida spp.</th>
<th>Sequence</th>
<th>Gene name</th>
<th>GenBank no.</th>
<th>Amplified size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>alBF</td>
<td>GCTGGCATATACCTGTGATTG</td>
<td>SAPS</td>
<td>AF043548</td>
</tr>
<tr>
<td></td>
<td>alBR</td>
<td>CGACCTTGGCATTGGAATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dubF</td>
<td>GGTCTATCTTATTTGCTAC</td>
<td>HWPI</td>
<td>AJ632273</td>
</tr>
<tr>
<td></td>
<td>dubR</td>
<td>CTCGAGGCGATTTCTGATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>glaF</td>
<td>ATGTCACCTGAAAACCTCTTGG</td>
<td>ERG11</td>
<td>L40389</td>
</tr>
<tr>
<td></td>
<td>glaR</td>
<td>CTGTCCTTTCACGGAAAATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>guiF</td>
<td>GATCCACACAGAACATTACGATG</td>
<td>XYL1</td>
<td>DQ297454</td>
</tr>
<tr>
<td></td>
<td>guiR</td>
<td>CATGACTAAAATGACACAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>kruF</td>
<td>ACCTGTATCCAGTGTCTAC</td>
<td>ABC1</td>
<td>DQ903906</td>
</tr>
<tr>
<td></td>
<td>kruR</td>
<td>CTGTCGGTGATGCGTGCTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>parF</td>
<td>GCTTTGGATGTGCTATTCG</td>
<td>rCR</td>
<td>DQ250667</td>
</tr>
<tr>
<td></td>
<td>parR</td>
<td>GCGCAATTCCTAAATGCGCAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>troF</td>
<td>GGCACGCGGATGTTGCAATTAATC</td>
<td>ACT1</td>
<td>AJ257918</td>
</tr>
<tr>
<td></td>
<td>troR</td>
<td>CCGATCTCAGAATACTTTCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
centrifuged at 10,000 rpm for 2 min, followed by heat treatment for 10 min at 70 °C. One μl of the supernatant was used as template DNA. Seven μl of a mixture of 7 pairs of primers (10 μM each, Table 1), 10 μl of Master Mix (GoTaq Green Master Mix, 2×; Promega KK, Tokyo, Japan), and 2 μl of distilled water were mixed to yield the multiplex PCR reaction solution at a gross quantity of 20 μl. PCR was performed by initial denaturation at 94 °C for 4 min, followed by 40 cycles at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 2 min, and a final extension at 72 °C for 7 min. Typical results of multiplex PCR using the above primer set are shown in Fig. 1. One specific band was observed for each of the 7 strains. The specificity of the primer set was confirmed by comparison with other standard strains, including negative control strains.

2.5. Questionnaire survey

Questionnaire items included age, gender, body mass index (BMI) [16], pedometer use, oral condition, hospital visits, medication, dental visits (once/year or more), and oral care (tooth brushing: times per day, brushing methods), exercise habits, alcohol habits, and smoking. The questionnaire was partially carried out via selection from prepared answers [gender, pedometer use/[or not], good oral condition/[or bad], alcohol habit [yes or no], smoking (only yes or no, regardless of smoking history), hospital visit (frequency) medication (yes or no), dental visit (frequency), and partially carried out via writing (BMI, oral care, exercise habits). Specific methods such as the Fortune, Stillmas, and Bass methods, which are known as widely accepted as reliable methods for the removal of food particles between teeth [17], were also adopted in this study as reliable methods. We judged the quality of exercise habits, by asking whether the participant had performed exercises according to the recommended guidelines of the Society of Japanese Physical Fitness and Sports Medicine. For example, we asked whether or not the number of steps/day exceeded 7000, and whether or not the exercise was undertaken at a sports club. Details regarding medications and hospital visits could not be surveyed in this study, due to juridical constraint, according to the Personal Information Protection Law (Japan).

2.6. Analysis

We set standards for each item in order to investigate the background factors underlying oral candidal carriage by statistical analysis. Criteria to judge non-appropriate health conditions were defined as follows: BMI < 18.4 or ≥25.1, regular hospital visits, regular medication (prescription by a clinical doctor), pedometer non-use, poor oral condition (having diseases such as dental caries and periodontal diseases), alcohol habit, smoking habit, no ongoing physical training, dental visit less than once a year, and improper tooth-brush execution. After calculating the percentage of the oral candidal carriage in the population of non-appropriate health conditions (non-appropriate population), candidal carriage in appropriate/non-appropriate were analyzed by the two-way layout ANOVA method (Fisher test).

A logistic regression analysis was performed with oral candidal carriage set as the independent variable, and each of the items set as a dependent variable. First, the ratio of candidal carriage among the non-appropriate population was examined for each item. The background factors underlying oral candidal carriage were then examined by multiple logistic regression.

2.7. Statistics

Fisher’s exact test and the Cochran Armitage test were performed, adopting a significance level of 5% (p-value < 0.05) [18].

A logistic regression analysis was performed to assess the association between the presence of Candida spp. and other items using statistical software (Eksusu-Toukei 2010; SSRI Co., Ltd., Tokyo, Japan). The relationship between the background factors (age, gender, BMI, pedometer use, oral condition, hospital visit, medication, dental visits, oral care, exercise habits, alcohol habits, and smoking) and candidal carriage of the target population was analyzed by a multiple logistic regression model. A stepwise method was used for variable selection.

2.8. Ethics

The survey was conducted in accordance with the provisions of the Ethics Committee of NDUN, and was undertaken with the participants’ consent.

3. Results

Among a total of 494 entries, 482 were analyzed after the deletion of entries who had not answered the questionnaire, and/or who had not accepted fungus test administration. The data incorporated in this study were obtained from 482 healthy volunteers aged from 20 to 60 years (including 263 in the 2011-survey, and 219 in the 2012-survey). Participants consisted of 269 healthy males aged 35.9 ± 14.0 years and 213 healthy females aged 33.0 ± 11.2 years. Number and age of volunteers are shown in Table 2.

3.1. Detection of Candida spp.

Candida spp. were detected via culture (containing antibiotics) of samples obtained from each subject’s tongue. The prevalence of Candida spp. in the oral cavities of the subjects was 18.3% (Table 3). The test result for the first survey was 19%, while that for the second was similar, at 17.4% (details not shown). No significant differences in gender were observed in oral candidal carriage (male, 17.8%; female 18.8%; Table 2). Carriage of Candida spp. was observed to decrease with age (Table 3) (p < 0.01; Cochran Armitage test).

Table 2
Summary of volunteers participated in this study.

<table>
<thead>
<tr>
<th>Number of participants (n)</th>
<th>Age (years ± SD)</th>
<th>Oral candidal carriage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>269</td>
<td>35.9 ± 14.0</td>
</tr>
<tr>
<td>Female</td>
<td>213</td>
<td>33.0 ± 11.2</td>
</tr>
<tr>
<td>Total</td>
<td>482</td>
<td>34.6 ± 12.9</td>
</tr>
</tbody>
</table>

No significant difference was observed in the oral candidal carriage between genders.

Table 3
Prevalence of Candida in the oral cavity of healthy volunteers.

<table>
<thead>
<tr>
<th>Candidal carriage by age</th>
<th>20s</th>
<th>30s</th>
<th>40s</th>
<th>50s</th>
<th>60s</th>
<th>AU</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive no./total</td>
<td>53/234</td>
<td>18/102</td>
<td>6/53</td>
<td>7/56</td>
<td>1/32</td>
<td>3/5</td>
<td>88/482</td>
</tr>
<tr>
<td>%</td>
<td>22.6</td>
<td>17.6</td>
<td>11.3</td>
<td>12.5</td>
<td>3.1</td>
<td>6.0</td>
<td>18.3</td>
</tr>
</tbody>
</table>

AU: age undetermined; Age dependency: p < 0.01 (Cochran Armitage test).
3.2. Identification of Candida spp.

In the PCR results for culture positive specimens, *C. albicans* accounted for approximately 80% of the species (Table 4). The ratio of *C. albicans* in positive colonies in the first survey was 82%, while that in the second survey was similar, at 79% (details not shown). In addition, the proportion of *Candida* species other than *C. albicans* was similar for the two surveys.

### Table 4
Number of participants positive for oral *Candida* species identified by multiple PCR.

<table>
<thead>
<tr>
<th>Species</th>
<th>Positive participants (n)</th>
<th>Positive % (n/total 482 participants)</th>
<th>Species distribution (n/total 88 positive participants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>71</td>
<td>14.73</td>
<td>80.7</td>
</tr>
<tr>
<td><em>C. dublinensis</em></td>
<td>5</td>
<td>1.04</td>
<td>5.7</td>
</tr>
<tr>
<td><em>C. guillermondii</em></td>
<td>6</td>
<td>1.25</td>
<td>6.8</td>
</tr>
<tr>
<td><em>C parapsilosis</em></td>
<td>3</td>
<td>0.63</td>
<td>3.4</td>
</tr>
<tr>
<td>Other sp</td>
<td>3</td>
<td>0.63</td>
<td>3.4</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>18.3</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 5
Difference in candidal carriage in appropriate and non-appropriate population for items.

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Non-smoking</th>
<th>Non-exercise habits</th>
<th>Exercise habits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier</td>
<td>18</td>
<td>70</td>
<td>59</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>41</td>
<td>353</td>
<td>211</td>
</tr>
<tr>
<td>Ratio of carrier (%)</td>
<td>30.5</td>
<td>19.8</td>
<td>27.9</td>
</tr>
<tr>
<td>p</td>
<td>0.018</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

Fisher test.

### Table 6
Association between the presence of oral *Candida* sp and the items after sorting – Logistic analysis: Adjusted odds ratio and 95% CI.

<table>
<thead>
<tr>
<th>Item</th>
<th>Odds ratio</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.96</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>Smoking habits</td>
<td>1.93</td>
<td>1.00</td>
<td>3.69</td>
</tr>
<tr>
<td>Exercise habits</td>
<td>1.84</td>
<td>1.10</td>
<td>3.08</td>
</tr>
</tbody>
</table>

3.3. Analysis

To investigate the background factors underlying oral candidal carriage, we employed the set of standards described in Section 2 for each item. First, we examined the candidal carriage ratio among non-appropriate population for each item. The resulting oral candidal carriage percentage in the non-appropriate population for each item was as follows: BMI, 18%; hospital visit, 20%; medication, 21%; pedometer, 19%; oral condition care, 23%; alcohol habit, 19%; smoking habit, 31%; exercise habits, 28%; dental visits, 22%; and tooth brush, 19%. When the difference between oral candidal carriage in non- and appropriate population was examined via Fisher’s exact test, significant differences were obtained for two items (smoking habit, non-exercise habit) ($p < 0.05$; Table 5). The involvement of age in candidal carriers with smoking habits was not observed (data not shown).

A logistic regression analysis was then performed, with oral candidal carriage set as the independent variable, and each of the items set as a dependent variable. When variable selection was performed by the stepwise method (cut off value, 0.05), age, exercise habits, and smoking were chosen as the model variables. The adjusted odds ratio (95% CI) for exercise habits was 1.84 (1.10–3.08), that for smoking was 1.93 (1.00–3.69), and that for age was 0.96 (0.94–0.98), respectively (Table 6). These results presented here strongly suggest that exercise habits and smoking habits are associated with oral candidal carriage.

4. Discussion

The aim of this study was to investigate the carriage rate of oral *Candida* spp. in healthy volunteers (dental students and staff) and to investigate the background factors underlying carriage. The carriage of *Candida* spp. in the oral cavities of participants was 18.3%. Notably, the percentage of oral candidal carriage obtained for students in their 20s was 22.6%, which was similar to that obtained in our previous survey undertaken 15 years earlier, which indicated a student carriage percentage of 30%. In fungi identification, 80.7% of culture positive specimens were identified as *C. albicans* in this study. While, 84–88% of them were *C. albicans* in a previous study using RVID Panel, which consisted of 27 substrates for testing the presence of produced enzymes [11]. However, as *C. dublinensis* was not known in those days, the possibility of misidentifying *C. albicans* for *C. dublinensis* cannot be ruled out. Therefore, the rate of *C. albicans* carriage in the previous study might be slightly higher.

Although oral candidal carriage showed a tendency to decrease with age, this is thought to be due to a decrease in elderly persons: 40’s (11.3%), 50’s (12.5%), and 60’s (3.1%). An increased prevalence of *Candida* sp. in the oral cavities of adults and very young population has been reported [3,13]. The increase in the neonatal period is probably due to the increased vulnerability that accompanies an immature immune system and an underdeveloped oral microflora [19]. Lockhart et al. reported that the frequency, intensity, and multispecies carriage of *Candida* increased with age (groups: 60–69 years, 70–79 years, and more than 80 years), possibly due to a weakening of the natural suppression of yeast carriage in the oral cavity in the elderly, which is in contrast to results obtained in this study [20,21]. Moreover, they showed that the intensity of candidal carriage (colony formation units; CFU) increased in an age-dependent manner and/or with denture usage (changes associated with decreasing oral condition). Because the low intensity candida carriage group (1–14 CFU) in their study seems to account for a relatively minor percentage of aging subjects, this discrepancy may be due to the distinctive characteristics of the subject group in this study, which comprised dental students and staff, who generally exhibit a greater level of concern regarding their oral health relative to the general public, as noted in the following discussion.

The prevalence of oral candidal carriage in the group of experts was lower than that of students, beyond our expectation. These experts were primarily composed of allied health occupation professionals with no clinical signs of xerostomia, who were concerned about their health in general, and oral health particular, on a daily basis. This result may be due to good oral care in experts. The presence of smoking habits was observed with high frequency in the younger population, particularly among students, and decreased with age. Moreover, the exercise habits in students were poor, compared with those of experts. We presumed that the high frequency of candidal carriage in younger participants was the result
of poor day-to-day health management. Therefore, it is evident that the importance of continuing oral care has been demonstrated by dental professionals.

Recently, a connection between BMI and oral conditions has been reported [22]. BMI was classified into three categories, as follows: overweight (BMI ≥ 25.1), acceptable weight (BMI > 18.5–25), and underweight (BMI ≤ 18.4) [WHO, 1995]. We attempted to analyze the relationship between BMI and oral candidal carriage using these 3 weight-categories, separately, although a clear relationship between these categories was not obtained. These results suggested that a BMI deviation, either a rise or fall, may facilitate oral candidal carriage. However, a relationship between the carriage of Candida spp. and exercise habits and smoking was observed in this study. It is known that exercise habits and smoking can affect BMI [23,24]. There is also the opinion that athletes, who participate in daily exercise, have a tendency to consume substantial amounts of carbohydrates [25], which may influence the outgrowth of Candida spp. However, in general, carbohydrate metabolism in athletes is known to be enhanced during exercise. Moreover, we must consider the oral cleaning effects of the water or sports drink that they take after exercise. Therefore, exercise habits may tip the balance in their favor, in terms of protection from oral candidal carriage.

A recent article reported that a lower frequency of toothbrushing was suggested to be associated with metabolic syndrome [26]. However, subjects with low tooth-brushing frequency were not encountered in this study: 21 of 482 participants indicated a tooth-brushing frequency of 1/day, while the remainder indicated a frequency of 2/day or more (details not shown). By contrast, it is interesting to examine the candidal carriage of persons who have neglected oral care.

5. Conclusion

We can at least confirm that exercise habits and smoking are involved as background factors in oral candidal carriage. Since these factors are already known relating ill health, the carriage of Candida spp. in the oral cavity of an individual appears to imply that they may not be in a healthy condition, even if no symptoms are present. In other words, being Candida free in the oral cavity may be requisite for the maintenance of good health, as oral candidiasis can never form without carriage. Thus, the oral carriage of Candida may serve as a health warning.

On the other hand, the discovery of the fungal side factors has not yet been undertaken, and remains an issue for future study.

Acknowledgment

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References