Correlations between oral *Candida* carriage and oral status in the healthy elderly

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Abstract - *Objective:* The aim of this study was to investigate the prevalence of *Candida* carriage and the distribution of *Candida species* and their correlations with oral status in healthy elderly. *Methods:* A total of 366 subjects aged 75 years participated in a questionnaire survey and oral examination. *Candida* specimens were collected from tongue dorsum and cultured on CHROMagar medium. *Candida species* was distinguished according to their colony morphologies and colors. *Results:* The result showed a high *Candida* carriage rate (65.3%) in the subjects and the predominant species was *Candida albicans* (50.8%), followed by *Candida glabrata* (26.2%). In *C. albicans* isolates, genotype A predominated (56.4%) and genotype D showed a higher prevalence (11.6%) than previous reports. When comparing *Candida* carriage with oral status, significant positive correlations were found with the presence of dental prostheses, missing teeth, the number of roots remaine and the percentage of sites showing positive bleeding on probing (BOP). Significant negative correlations were found with the number of teeth present, sound and filled teeth. Poor oral conditions and a reported allergy were significantly correlated with *Candida* carriage in the questionnaire. *Conclusions: Candida* carriage is significantly associated with oral status, especially the presence of dental prostheses. *C. albicans* and its genotype A were predominant and *C. glabrata* was relatively higher in this healthy elderly group.

Key words: *Candida species*; the elderly; oral status; epidemiology;

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The growing interest in oral infections caused by *Candida species* is related to the occurrence

of HIV-infection and the more widespread use of immunosuppressive chemotherapy (1, 2).

Candida species are frequently encountered as harmless and a part of the normal human

microbial flora in oral cavity (3). The carrier may show no sign of oral mucosal disorders.

However, some carriers with a high density of *Candida species* may become infected as oral

Candidosis which has a higher prevalence in the elderly and may prompt the dentist or

clinician to look out for underlying pathology changes in the seemingly healthy individuals.

Since *Candida species* differ both in the putative virulence factors and sensitivity to

antifungal agents (4, 5), it is important to identify the isolates by *Candida species*.

CHROMagar is a very useful medium to identify *Candida* species: *C. albicans, C. tropicalis,*

C. krusei, C. glabrata, and C. parapsilosis which accounted for almost 90% of the clinical

yeast isolates (6). However, CHROMagar can not definitely distinguish *C. albicans* from *C.*

dubliniensis (7, 8) which is a newly identified strain of *Candida species* in a HIV positive

patient. Therefore, the polymerase chain reaction (PCR) method was employed to determine

intra-species genetic heterogeneity including the differentiation of *C. albicans* from *C.*

dubliniensis. With the application of PCR methods, it is possible to make a more sensitive and

rapid study on *Candida* identification not only for individuals, but also for epidemiologic

surveys at genotype levels. In recent years, there have been more reports presenting the results on *Candida* status in hospitalized group or groups with general diseases such as HIV, carcinoma or diabetes (9-12). Although these infections occur in the healthy elderly as well, however, there is little information on *Candida* carriage and its distribution using advanced identification methods. Therefore, the purpose of this study was 1. to determine the prevalence and density of oral *Candida* carriage status in the healthy elderly 2. to investigate the distribution of *Candida species* and the genotypes of *Candida albicans* in this group of subjects 3. to investigate the correlations between *Candida* carriage and oral status.

Materials and methods:

Subjects

This study is a part of a comprehensive longitudinal study to evaluate the oral and general conditions of healthy Japanese elderly. The subjects were recruited from people born in 1927, currently residing in the city of Niigata, Japan. In the comprehensive study, a questionnaire survey, oral and general health examinations were conducted on the same day in local community centers in Niigata city. In this study, only the questionnaire survey and oral status were analyzed and compared with *Candida* carriage. A total of 366 subjects (aged 75) who had been followed up for five years participated in this study. None of the subjects was hospitalized or institutionalized. They were systemically healthy and did not require special care for their daily activities. Informed consent was obtained from all subjects prior to this study.

A questionnaire was completed by a personal interview to obtain the bulk of information regarding gender, smoking and alcohol drinking habits, utilization of dental services and dental self-care behaviors, etc. Oral examinations were carried out by four dentists who were well calibrated on volunteer patients in the Niigata Faculty hospital before the survey. Under sufficient artificial light, the oral examinations were conducted by using dental mirrors and specially designed periodontal probes (Vivacare PTS Probe®). For caries status, DMFT on both crown and root were recorded. Probing was performed at six sites for all the teeth, and bleeding on probing (BOP), pocket depth (PD) and loss of periodontal attachment in millimeters (LA) were recorded for periodontal status. Prosthetic status was recorded as follows: bridge, crown, partial denture and complete denture. The cleanliness of the denture was recorded at the same time. In addition, the condition of oral mucosa was examined for all subjects as well.

Candida specimens

The *Candida* specimens were collected from the dorsum of tongue by swabbing ten times with a sterile cotton stick and immediately inoculated on a selective medium for *Candida*, CHROMagar™ Candida (CHROMagar, Paris, France). *Candida species* were distinguished by their colony morphologies and colors on the plates. According to the manufactory chart, *C. albicans* isolates show distinctive green color, *C. glabrata* in purple to pale pink, *C. tropicalis* in dark blue with pinker edge, *C. krusei* in rough spreading colonies with pale pink centers

and a white edge, and *C. parapsilosis* in white or pale pink. After aerobical incubation for 48 hours at 30 °C, the numbers of colony with the same species were counted and expressed as colony forming units (CFU) per swab for each *Candida species* respectively.

Genotyping of Candida albicans

Extraction of DNA: One isolate colony of *Candida albicans* from each plate was selected and sub-cultured on Sabouraud agar medium. One loopful of *Candida* cells were suspended in 200 μm of PrepMan™ Ultra Reagent (Applied Biosystems, CA) and mixed with votex, followed by boiling for 10 minutes at 100 °C. The aqueous phase was separated by centrifugation at 12,000xg for 3 minutes and the suspension solution was used as DNA template in PCR amplification.

Genotype of *Candida albicans* was determined by PCR as genotype A, B, C, and D (*C.dubliniensis*) (13). The PCR primer pairs were the sequences span the site of the transposable intron in the 25S rDNA and as follows: CA-INT-L:5'-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3' and CA-INT-R:5'-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3'DNA. A 50 μl PCR reaction volume consists of 1 μl of DNA template. The PCR conditions were as follows: samples were denatured by incubation for 15 minutes at 95 °C before 30 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 2.5 min, and a final extension at 72 °C for 8 min in a PCR 9700 device (Applied Biosystems, CA). All amplification products were analyzed by electrophoresis in 1xTAE buffer on 1.0% agarose

gels mixed with 0.1μg ethidium bromide per ml and visualized under ultraviolet light. The approximate PCR product lenghs (bp) for genotype A, B, C, and D are 450, 840, 450 plus 840, and 1080, respectively. *C. albicans* reference strains (*Candida albicans* serotype A – ATCC90028) were employed and confirmed with above methods.

Statistic analysis

All the data were computerized and analyzed using the Stata Release 7.0 statistical package (14). Student's t-test and ANOVA were performed to identify the differences between two or more groups in CFU value. Chi-square test was used to test the difference in prevalence. Pairwise correlation coefficients with Bonferroni adjustment significance level were calculated to investigate the associations between *Candida* values and each of the oral parameters used. As prosthetic status might be highly associated with the presence and density of *Candida* colonization (15), an analysis was performed to compare those subjects with and without a prosthesis.

Results

No visible plaque was observed on the partial dentures or dentures during the oral examinations. No obvious mucous disorders were found as well of all the participants. The general information of *Candida spp.* and prosthetic status in male and female is presented in Table 1. About sixty-nine percent of the total subjects carried *Candida species*. The oral carriage of *Candida spp.* was significantly higher both in frequency and density in female

than those of male. Female was also significantly higher in having prostheses than male.

Table 2 shows the frequency and distribution of subjects with single and multiple *Candida* species. The numbers in bold were the subjects with sole species of *Candida*. The predominant *Candida* species is *C. albicans* (50.8%), followed by *C. glabrata* (26.2%) and *C. parapsilosis* (5.7%). There were 6.6 % of subjects carried colonies which could not be clearly identified their *Candida* species by CHROMagar medium. So these colonies were classified into the group as 'others' of *Candida* species. In 186 *C. albicans* positive subjects, 175 (94.1%) had carrying at least one kind of prosthesis, of which 137 (73.7%) were partial or full dentures. About Sixty-eight percent of *C. glabrata* harbored predominantly together with *C. albicans* and twenty-four percent remained as sole species. There were significant differences (p<0.01) in the presence of *C. albicans* and *C. glabrata* with having a prosthesis or not.

Most of non-*C. albicans* emerged as a multispecies carriage and occurred mainly in subjects with prostheses. The percentage of carrying 1 to 4 kinds of *Candida* species was 43.2%, 18.6%, 6.0% and 0.8%, respectively. The three subjects who carried four kinds of *Candida* species were all wearing a partial or complete denture. Of those carried three kinds of *Candida* species, 16 out of 22 subjects (72.7%) had a partial or complete denture.

Table 3 presents the distribution of *C. albicans* by genotype in subjects with and without a prosthesis. Genotype A predominated in the total of 181 isolates, followed by genotype C, B and D. Genotype D (21 isolates) was identified only in the group of having prostheses. A

significantly higher percentage of Genotype A was found among the four genotypes when a prosthesis was present. There was no difference in the distribution among four genotypes in subjects without a prosthesis.

The number of dentate and edentulous was 336 (91.8%) and 30 (8.2%) respectively in this group of subjects. The *Candida* carriage status by detailed prosthetic condition is shown in Table 4. The mean number of *Candida spp.* counting increased from 9.4 to 70.3 CFU with an increasing complexity of prostheses and the differences were significant using the ANOVA test adjusted by Bonferroni significant level. The percentage of *Candida* carriage also significantly increased from 32.6 % to 78.1%. The odds ratio of the *Candida* carriage status by prosthetic conditions was 3.0 which means those who wear prostheses have three times the risk of carrying *Candida* than those without any prosthesis. There was no significant difference in the mean CFU of *Candida* with complete denture on either jaw.

As shown in Table 5, males have significant higher sound teeth, root caires and wedge shape defect (WSD), but greater PD and LA in periodontal conditions. However, females revealed significantly better treatments, more crown and filled teeth as well as more prostheses. The mean values in each variable were used to conduct the pairwise correlation coefficient with *Candida* status. Significant positive correlations were observed in the number of missing teeth, roots remain, the percentage of BOP positive sites, and the presence of any kind of prosthesis. While significant negative correlations were found in the number of teeth

present, sound and filled teeth as well as the presence of WSD both filled and unfilled.

All the variables in questionnaire were analyzed, however, only those which might relate to oral *Candida* carriage and those having significant correlations were shown in Table 6. Burning tongue, pain from denture, current status of teeth and gums, mouth ulcers, allergies, eating snacks and frequency of eating snacks were all significantly positively correlated with *Candida species*. No significant correlations were found in dry mouth, mouth breathing, bad breath and smoking.

Discussion

Accurate data for *Candida* carriage rates in healthy individuals can only be obtained if the clinical status of each subject tested is carefully and expertly assessed. Many precious studies published contained little information about the general, intra-oral and clinical status of healthy individuals. In this study, the participants were selected from a group of elderly who had not attended hospital for any treatment and were judged to be relatively healthy by the general results from the health examinations on the same day. The higher mean number of teeth present in the oral cavity (17.1) revealed relatively better oral conditions in this group of subjects compared with the reports by Ministry of Health and Welfare in Japanese (11.0) at the same age of 75 (16).

Although the frequency of yeast carriage in oral cavity was dependent on isolation

techniques and time of sampling, the median carriage frequency was 34.4% for all yeast and

17% for *C. albicans* (4). Scully et al. (17) reported a higher percentage of *C. albicans* isolated

rate (47-84%), followed by *C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei.* The

results in this study were similar to the above reports with the *C. albicans* (50.8%)

predominant, but with a higher isolated rate of *C. glabrata* (26.2%). Odds (4) stated *C.*

glabrata is often found in significant numbers, with the highest frequency in denture wearers

and those with denture-induced stomatitis. Luo (18) reported that both *C. albicans* and *C.*

glabrata had significant positive correlations between cell surface hydrophobicity(CSH) and

adhesion. Compared with *C. albicans*, *C. glabrata* demonstrated a four-fold greater CSH and

a two fold greater tendency to adhere to denture acrylic surface. This might be one of the

reasons that *C. glabrata* occurred more often accompanying by *C. albicans*, especially when

there is a prosthesis in the oral cavity.

With the presence of a prosthesis, not only the total *Candida* counts but also its species

increased. MacFarlane (19) stated that approximately 10% of oral samples harbor more than

one *Candida spp.* This study presented a higher percentage (25.4%), of which 96.8%

possessed at least one kind of prosthesis. Therefore, the presence of prosthesis, not only

partial or full dentures but also bridges, could be a key factor in healthy individuals who carry

a higher amount and more variety of *Candida spp.* although there are many other factors

related to oral *Candida* carriage.

C. dubliniensis is a newly recognized species of *Candida* and is phenotypically very similar to *C. albicans* (20, 21). In 1995, this species was first isolated from the oral cavity of HIV positive patients with candidosis. Later on *C. dubliniensis* was detected not only in HIV-positive subjects but also in healthy individuals (8, 22, 23). On CHROMagar™ Candida plate, both *C. albicans* and *C. dubliniensis* formed greenish colonies, which can only be distinguished from other non-*Candida albicans*. Although Coleman et al.(22) reported that *C. albicans* colonies appear light green in color, in contrast, *C. dubliniensis* appear dark green, it was confirmed that the colonies yielded on CHROMagar Candida medium can be unstable following subculture or storage (7, 8).   Recently, a PCR method has been used to classify *C. albicans* strains into four genotypes on the basis of the amplified PCR product length: genotype A (450-bp), genotype B (840-bp), genotype C (450- and 840-bp) and genotype D (1080-bp), which genotype D was also confirmed to belong to the same taxon as *C. dubliniesis*. The majority of subtypes identified in this study were genotype A (56.4%), which was similar to the report (57.1%) by Tamura et al (8) on the basis of 301 clinical isolates of *C. albicans* in Japan. However, a higher percentage (11.6%) of genotype D was identified in this study compared with 1.7% in the above report. As *C. dubliniensis* was first and more often

identified from oral specimens, the origin of specimens might be the reason which accounted for it in this study. It was interesting that all the subjects who harbored *C. dubliniesis* were wearing at least one type of prosthesis. However, further studies are needed to confirm if this trend is a common or chance finding. Despite the many similarities between *C. dubliniesis* and *C. albicans*, there are significant differences between the two species (4). The former has the ability to rapidly develop fluconazole resistance (24) which has clinical implications for antifungal drug treatment regimens.

Many studies have also reported that the *Candida* carriage rate varies with gender. Some reported a higher *Candida* carriage rate in female than in male (25, 26). However, no explanation was given. In 1999, Lockhart et al. (27) reported a higher carriage rate for male than for female in the frequency and intensity of carriage, and the multiplicity of species. The latter also stated that the above trends could only be found for individuals without dentures. From the reports (10, 15), the subjects wearing dentures had a greate number of *Candida* than those who did not. The results of this study (Table 4) also showed that wearing a prosthesis could strongly affect the *Candida* carriage. Thus, the higher prevalence of prostheses in females than that in males could be one of the main reasons for the higher *Candida* carriage rate in elderly females in this study.

As the fewer teeth present in a subject, the more likely there is to be a prosthesis, therefore

significant correlations could be found in those variables as shown in Table 5 with *Candida* status. In periodontal conditions, only BOP was significantly correlated with the carriage of *Candida*. Periodontal diseases are caused by the interaction between micro-organisms and the hosts immune system in the periodontal environment (28). Reynaud et al (29) reported that *Candida* was a frequently occurring micro-organism in the periodontal microbial flora. BOP indicates inflammation and these changes in oral environment might favor the growth of *Candida* as well as other organisms in patients with periodontitis.

In the questionnaire, worse oral conditions were significantly correlated with the higher carriage of *Candida,* which is in agreement with many reports (10, 30). Although further analysis between general conditions and *Candida* carriage has not been conducted in this study, an allergy (a sign of systemic immune system disorder) was found to be highly correlated with the presence of *Candida* species. The oral cavity is a part of the secretory immune system which can be stimulated locally or systemically. Therefore, systemic immune system problems could affect the carriage of *Candida* in oral cavity. There is no consent yet on the relationship between cigarette smoking and oral *Candida* carriage (26, 31, 32). No significant correlation was found between smoking and *Candida* carriage in this study. Further carefully designed studies which include recording the presence of a prosthesis are needed to find a possible relationship.

In conclusion, not only dentures but also other prostheses could affect *Candida* carriage in

its frequency, intensity, multi-species and genotype of *Candida albicans*. The percentage of *C. glabrata* tends to increase and *C. dubliniesis* could be detected in healthy elderly who are wearing prostheses. The correlations between general health conditions and *Candida* carriages are still not clear. A carefully designed study is necessary to investigate a possible relationship in the healthy elderly.

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