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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 a 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.

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 quormones (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.

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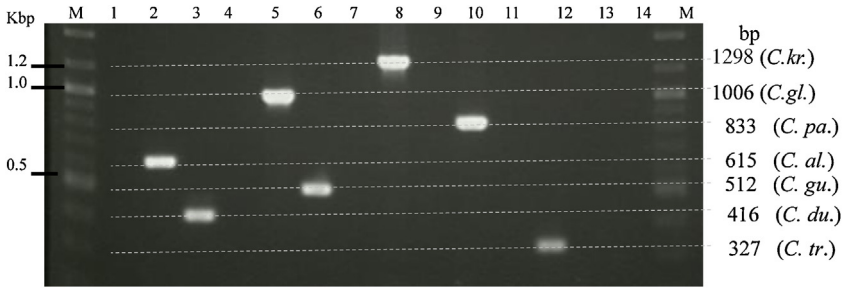

Fig. 1. Direct multiplex polymerase chain reaction of *Candida* 7 type strains for screening in 2% agarose. Lane M, marker (2-Log Ladder); lane 1, template minus (negative control); lane 2, *C. albicans* (IFM 40009); lane 3, *C. dubliniensis* (IFM 54665); lane 4, *C. famata* (IFM 55255); lane5, *C. glabrata* (IFM 54350); lane 6, *C. guillermondii* (IFM 54665); lane 7, *C. kefyr* (IFM 46842); lane 8, *C. krusei* (IFM 5462); lane 9, *C. lusitaniae* (IFM 52638); lane 10, *C. parapsilosis* (IFM 5804); lane 11, *C. pelliculosa* (IFM 5459); lane 12, *C. tropicalis* (IFM 46821); lane 13, *C. utilis* (IFM 40099), and lane 14, *Saccharomyces cerevisiae* (IFM 48491). These type strains were provided by Culture Collection of the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan. Each *Candida* species was identified by one clear, specific band.

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 Nippon 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* colonies were suspended in 50 μl of 25 mM NaOH. After freezing and thawing, the suspension was

Table 1
Primers used in multiplex PCR for identifying *Candida* species.

Identification of *Candida* spp.		Sequence	Gene name	GenBank no.	Amplified size (bp)
Candida albicans	**albF**	GCTCGCATATACCTGTCATTG	*SAP5*	AF043548	615
	albR	CGAGCTTGCCATTTGAATG			
C. dubliniensis	**dubF**	GGCTCATCTATTTTAGCTAC	*HWP1*	AJ632273	416
	dubR	CCTGGAGCCGATTCTGTAGT			
C. glabrata	**glaF**	ATGTCCACTGAAAACACTTCTTTG	*ERG11*	L40389	1006
	glaR	CTGGTCTTTCAGCCAAATGC			
C. guilliermondii	**guiF**	GATCCACAGGAACATTATCGATG	*XYL1*	DQ297454	512
	guiR	CATGACTAAAATGGACCAC			
C. krusei	**kruF**	ACCTTGATCCAGTTGCTTAC	*ABC1*	DQ903906	1298
	kruR	CTCGTGGTAGTCCTGGTTC			
C. parapsilosis	**parF**	GCTGTTGGATTGTGTCATTCTG	*rCR*	DQ295067	833
	parR	GGCAATTCCTTCAATTTGGCAC			
C. tropicalis	**troF**	GGACGGGGGTATGTTTCAATTAAATC	*ACT1*	AJ237918	327
	troR	CCGATTACAGATAAGTAATTTCC			