# Frequency and Distribution of *Candida* Species from Denture Wearers

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#### ABSTRACT

Species of Candida were isolated from 100 denture wearers, who were examined for grade of denture stomatitis, degree of denture plaque accumulation and other clinical features. Candida albicans was the dominant species isolated from denture surfaces followed by Torulopsis glabrata and Candida tropicalis. Statistical analysis of the results revealed close relationships between the grade of denture stomatitis and the degree of denture plaque accumulation or the fungal concentration on the denture surface, and also between the degree of denture plaque accumulation and fungal concentration on the denture surface. Furthermore, the grade of denture stomatitis correlated with the period of denture wearing but not with the patient's age. The fungal concentration on denture surface also correlated with the patient's age and the period of denture wearing.

A large portion of denture wearers exhibits chronic atrophic candidosis (denture stomatitis) on the mucosa under the denture. It has been suggested that species of Candida frequently isolated from denture surfaces cause denture stomatitis<sup>4-6)</sup>. Experiments with an animal model have shown the pathogenicity of Candida to the mucosa under the denture8,10). Fungi are more frequently isolated in denture wearers with denture stomatitis than in those without denture stomatitis<sup>1,3,9)</sup>. However, few investigations have been conducted on the relations between the occurrence of Candida species and various patient's attributes such as age, sex, period of denture wearing, degree of denture plaque accumulation and grade of denture stomatitis.

The present study examined the occurrence of *Candida* species in denture wearers with and without denture stomatitis and also the relations between denture stomatitis and the conditions of denture wearers.

### MATERIALS AND METHODS

Patients. One hundred denture wearers composed of 70 females and 30 males whose age ranged from 28 to 87 (mean 65.0) and their 179 dentures were examined in this study. Sixty-six of them were patients with denture stomatitis and 34 were without denture stomatitis. Patients with denture stomatitis felt to be caused by trauma were excluded from this study.

Questionnaire. The age, sex and period of denture wearing were recorded.

Clinical examinations. Patients were examined by a single dentist for the following:

- 1) grade of denture stomatitis; -: no symptom, +: slight inflammation, ++: moderate inflammation, +++: severe inflammation
- 2) degree of denture plaque accumulation; -: no visible plaque, +: slight, ++: moderate, +++: severe

Fungal culture and identification. The denture was washed with running water and the fungal probe was sampled from the fitting surface. this probe was inoculated on Candida GE (yeast extract 1.0%, peptone 0.85%, glucose 3.0% and nitroflan derivatives 0.05%, Nissui, Tokyo Japan) plate. After 24 hr incubation at 37°C, colonies were counted and some representative colonies (at least 2 colonies for each colonial type) were transferred to Sabouraud glucose (peptone 1.0%, yeast extract 0.5% and glucose 2.0%) slope and incubated at 37°C. After twenty-four hours incubation on Sabouraud glucose slopes, the isolates were identified serologically by Candida check (Iatron laboratories Inc., Tokyo, Japan). In 26 patients, whole saliva was collected and 0.1 ml of the aliquot was inoculated onto a Candida GE plate for measuring fungal concentration in the saliva. From colony counts of the denture swabs, the fungal concentration on denture surface was scored as follows: -: no colony, +: 0-250 colonies, +: 251-500 colonies. +++: over 501 colonies.

Statistical analysis. The distribution of the results was not normal and hence, non-parametric statistical analysis was employed to determine the significance level. Kendall's rank correlation coefficient and significance test or Mann-Whitney's U-test were used to analyze the results, as appropriate.

### RESULTS

Table 1 shows the prevalence of Candida species in denture wearers with and without denture stomatitis. Candida species were isolated from 91 patients. C. albicans A was recovered most frequently, followed by T. glabrate and C. tropicalis. A single species was isolated from 39 patients, and two, three and four species were isolated from 38, 10 and 4 patients, respectively.

Table 2 shows the relationships of the grade of denture stomatitis and fungal concentration on denture surface, to the degree of denture plaque accumulation, fungal concentration on denture surface, patient's age and period of denture wearing. The grade of denture stomatitis increased with increasing degree of denture plaque accumulation and fungal concentration on denture surface. The fungal concentration on denture surface is highly correlated with the degree of denture plaque accumulation. Furthermore, the grade of denture stomatitis elevated with increase in period of denture wearing. However, the grade of denture stomatitis was not correlated with the patient's age. Correla-

Table 1. Prevalence of Candida species in 100 denture wearers

Species	No. of patients		
C. albicans A	74		
C. albicans B	6		
T. glabrata	36		
C. tropicalis	17		
C. krusei	8		
C. parapsilosis	4		
C. guilliermondii	1		
unidentified	12		
not isolated	g		

tion of the fungal concentration on denture surface with the patient's age was significant (0.01 , and that with the period of denture wearing was highly significant <math>(p < 0.01).

Relationships of the degree of denture stomatitis and fungal concentration on denture surface to sex and location of the denture are shown in Table 3. No significant correlation was demonstrated between sex and the grade of denture stomatitis or the fungal concentration on denture surface. However, more advanced denture stomatitis was observed on the mucosa under the upper dentures than on the mucosa under the lower dentures, and the fungal concentration was higher on denture surface of the upper dentures than on that of the lower dentures, The difference was highly significant (p<0.01).

As shown in Table 4, a linear relationship was observed between the fungal concentration in the saliva and that on denture surface.

#### DISCUSSION

C. albicans was isolated from 80 of 100 denture wearers and T. glabrata, C. tropicalis and C. krusei were recovered from 36, 17 and 8 individuals, respectively. This distribution of Candida species resembled that observed by Olsen<sup>6</sup>. However, the percentage of patients with infection by a single species was smaller in our experiment. Olsen inspected 100 patients all with denture stomatitis, and about 15% of them were infected by a single species other than C. albicans. In our experiment, 10.6% of the patients with denture stomatitis were infected by a single Candida species other than C. albicans. These findings suggest than not only C. albicans but also other Candida species may be able to cause denture stomatitis.

Table 2. Relationships of the grade of denture stomatitis and fungal concentration on denture surface, to denture plaque accumulation, fungal concentration on denture surface, age and period of denture wearing

		Denture stomatitis			Fungal concn on denture				
		_	+	++	+++	_	+	++	+++
Denture plaque	_	48 <sup>a</sup>	9	5	1	27ª	24	6	6
accumulation	+	29	10	45	3	1	18	14	54
	++	4	3	16	4	0	4	2	21
	+++	1	0	1	0	0	0	0	2
			$\tau^{\rm e} = 0.4$	45 HS <sup>c</sup>			$\tau^{\rm e} = 0.5$	66 HS <sup>c</sup>	
Fungal concn	_	24ª	3	1	0				
on denture	+	26	5	15	0				
	++	8	5	7	2				
	+++	24	9	44	6				
			$\tau^{\rm e} = 0.8$	37 HS <sup>c</sup>					
Age	-50	$6^{\mathrm{b}}$	2	4	0	$5^{\mathrm{b}}$	0	3	4
	51-60	10	3	8	3	6	4	2	12
	61 - 70	7	5	13	1	0	6	5	15
	71-80	6	4	14	1	1	7	4	13
	81—	5	0	7	1	0	2	1	10
			$\tau^{\rm e} = 0.1$	12 NS <sup>e</sup>		$\tau^{\rm e} = 0.20  \mathrm{S}^{\rm d}$			
Period of	0-2mo	8 <sup>b</sup>	1	3	0	<b>4</b> <sup>b</sup>	3	1	4
denture wearing	2-4mo	2	2	0	0	2	0	2	0
· ·	4-6mo	5	1	4	0	1	5	3	1
	6mo-1yr	7	2	4	0	2	4	2	5
	1-2yr	4	2	9	1	2	3	2	9
	2—5yr	5	2	12	1	1	2	1	16
	$5-10 \mathrm{yr}$	1	4	9	3	0	1	3	13
	10yr—	2	0	5	1	0	1	1	6
			$\tau^{\rm e} = 0.8$	35 HS <sup>c</sup>			$\tau^{\mathrm{e}} = 0.4$	10 HS <sup>c</sup>	

anumber of dentures

<sup>b</sup>number of patients

Table 3. Relationships of the grade of denture stomatitis and fungal concentration on denture surface, to sex and location of denture

		Denture stomatitis			Fungal concn on denture				
		_	+	++	+++	_	+	++	+++
Sex	male	13ª	2	12	3	5ª	4	5	16
	female	21	12	34	3	7	15	10	38
		$Zcal^e = 0.41 NS^c$				$Zcal^e = 0.18 NS^c$			
Location	upper	$32^{b}$	14	45	6	11 <sup>b</sup>	18	15	53
	lower	50	8	22	2	17	28	7	30
		$Zcal^e = 3.39 HS^d$				$Zcal^e = 2.76 \text{ HS}^d$			

anumber of patients

bnumber of dentures

 $<sup>^{\</sup>rm c}{\rm HS}$ : Correlation is highly significant, p<0.01

<sup>&</sup>lt;sup>d</sup>S: Correlation is significant, 0.01<p<0.05

eNS: Correlation is not significant, p<0.05

 $<sup>^{\</sup>mathrm{e}}\tau$ : Kendall's rank correlation coefficient

<sup>°</sup>NS: Difference is not significant, p<0.05

<sup>&</sup>lt;sup>d</sup>HS: Difference is highly significant, p<0.01

eZcal: Mann-Whitney's Z calculation

Table 4. Relationship between the fungal concentration on denture surface and that in saliva

Fungal concn in saliva	Fungal concn on denture surface						
(CFU/0.1 ml)	_	+	++	+++			
0	2ª	1	0	0			
1 - 250	1	3	1	2			
251 - 500	0	2	1	2			
501—	0	1	1	9			

anumber of patients  $\tau = 0.60$ 

Correlation is highly significant, p<0.01

The data obtained by clinical examination were treated by non-parametric statistical analysis. since data of this kind were not normally distributed. The grade of denture stomatitis was highly correlated with the degree of denture plaque accumulation and the fungal concentration on denture surface. This finding has been reported by several investigators 4-6). However, most analyzed the data without statistical methods or did not use ordinal measurements even if analysis was conducted utilizing statistical methods. The grade of denture stomatitis was also correlated with the period of denture wearing. Although the fungal concentration on denture surface correlated with the patient's age, correlation between the grade of denture stomatitis and the patient's age was not significant. These findings suggest that the grade of denture stomatitis is affected by the age of denture rather than the changes in the oral conditions of the patient; including the type of oral microflora.

Although the number of female patients was more than twice that of male patients, sex difference did not affect the grade of denture stomatitis or the degree of denture plaque accumulation. Davenport reported a higher incidence of denture stomatitis in female patients than in male patients<sup>5)</sup>. The percent of patients with denture stomatitis was also higher in female (70.0%) than in male (56.7%) in this study, but the difference was not significant. A greater accumulation of denture plaque and a higher fungal concentration were observed in the upper dentures than in the lower dentures, and a higher grade of denture stomatitis was observed on the mucosa under the upper dentures than

on the mucosa under the lower dentures. Better stability of the upper denture than the lower denture may hamper the salivary flow under a denture and this circumstance provides a more suitable environment for fungal growth. The fungal concentration in the saliva highly correlated with that on denture surface. This fact suggested that the fungal concentration on denture surface could be followed by counting the fungal concentration in the saliva.

Many investigators have suggested the participation of *Candida* species in the initiation and progression of denture stomatitis<sup>4-6</sup>. Results from this study also support this suggestion. Furthermore, correlation of the period of denture wearing to the degree of denture plaque accumulation and grade of denture stomatitis was observed. It can be also concluded from this study that removal of denture plaque may be essential for the prevention of denture stomatitis, especially in the patients with long period of denture wearing.

Olsen introduced chlorhexidine and amphotericin B in the clinical treatment of denture stomatitis, and decrease of fungi and erythema score was observed during the treatment period<sup>7)</sup>. Bergendal et al. applied nystatin, an antifungal drug, to patients with denture stomatitis, and reported a decrease of fungal density and a decrease of erythema<sup>2</sup>). We examined twentyone enzymes for their ability to remove Candida species from acrylic surfaces. Among these enzymes, proteolytic enzymes and  $\beta$ -1, 3-glucanase, at a concentration which did not lyse cells, were found to be most effective<sup>11)</sup>. The application of these enzymes is also considered to be effective in removing Candida species from denture surfaces.

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