Assessment of *Candida* Species Colonization and Denture-Related Stomatitis in Bar- and Locator-Retained Overdentures

Kerem Kilic[1]*
Ayse Nedret Koc[2]
Fatma Filiz Tekinsen, DDS, PhD[2]
Pinar Yildiz, PhD[1]
Duygu Kilic, DDS, PhD[3]
Gokmen Zararsiz, DDS, PhD[4]
Erdem Kilic[5]

The aim of this study was to assess the prevalence of denture-related stomatitis (DRS) in different attachment-retained overdenture wearers and its association with particular colonizing *Candida* species. Thirty-seven edentulous patients with implant-supported maxillary or mandibular overdentures were enrolled. A full clinical history was obtained, including details of patients' oral hygiene practices and the levels of erythema based on Newton's classification scale. Swabs were taken from the palate and investigated mycologically to identify the yeast colonies. Quantitative and qualitative microbiological assessments were performed, which included recording the total numbers of colonies (cfu), their color, and their morphological characteristics. Significant differences were found in cfu values between the attachment and inner surfaces of locator- and bar-retained overdentures ($P < .05$). *Candida albicans* was the most common species in both evaluations, being isolated from 81.3% of bar-retained overdentures and 38.1% of locator-retained overdentures. DRS developed in all patients using bar-retained overdentures but in only 71.4% of those using locator-retained overdentures. No statistically significant relationship was found between bar and locator attachments according to smoking habit, overnight removal, or plaque and gingival indices ($P > .05$).

Key Words: Candida species, denture-related stomatitis, overdenture

INTRODUCTION

Denture-related stomatitis (DRS) is a common inflammatory process that mainly involves the palatal mucosa when it is covered by complete dentures.[1,2] The etiology appears to be multifactorial; old age and the associated decline of immune defenses, systemic diseases, continuous denture wearing, increased age of denture, and lack of denture cleanliness resulting in the accumulation of plaque on the denture have all been proposed as predisposing factors.[3–6]

Besides the above-mentioned predisposing factors, several studies have demonstrated an association between *Candida* species and DRS.[7–12] Denture-related stomatitis is a disease of fungal and bacterial origin, and *Candida albicans* is the most frequently isolated yeast from the oral cavities of these patients.[7,8] In recent years, various studies have isolated other *Candida* species that may be involved in the pathogenesis of patients with oropharyngeal candidosis.[13–15] A wide variety of organisms has been shown to cause infection, with *Candida glabrata*, *Candida dubliniensis*, *Candida*

[1] Department of Prosthodontics, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.
[2] Department of Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
[3] Department of Periodontology, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.
[4] Department of Statistics, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
[5] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.
* *Corresponding author,* e-mail: dtkeremkilic@hotmail.com
DOI: 10.1563/AAID-JOI-D-12-00048

parapsilosis, *Candida krusei*, and *Candida tropicalis* being the most commonly described.[16]

Candida colonization and biofilm formation on dentures can be further affected by the species of colonizing *Candida*, oral hygiene practices, and denture characteristics.[17,18] Personal hygiene factors, such as denture removal at night, denture cleanser use, and smoking, have been shown to affect colonization and DRS.[19,20]

The retention of complete dentures by means of oral implants is a therapy widely appreciated by both patients and practitioners. Various methods to connect overdentures and implants have been described. Stud (locator, ball) and bar attachments are frequently used.[21,22] Several studies have examined possible differences between these attachment options in terms of denture stability and retentive forces, peri-implant attachment loss, financial aspects of treatment, degree and frequency of long-term complications, patient satisfaction, and clinical peri-implant findings.[21–25]

To our knowledge, no study to date has adressed the *Candida* species related to DRS in patients with different overdenture attachments. The aim of this study was therefore to assess the prevalence of DRS in wearers of different attachment-retained overdentures and its association with particular colonizing *Candida* species. We also aimed to evaluate the impact of patients' smoking habits, prosthesis usage habits, and awareness of prosthesis hygiene. The null hypothesis of this study was that no difference between locator- and bar-retained overdentures would be found in the prevalence of DRS and colonization by *Candida* species.

Materials and Methods

Patients

Thirty-seven edentulous patients with implant-supported maxillary or mandibular overdentures, who had scheduled their annual recall visit and had provided written informed consent, were asked to participate in the study. Of these, 16 patients wore bar-retained prostheses (10 mandibular, 6 maxillary), and the remaining 21 patients wore locator-retained prostheses (18 mandibular, 3 maxillary). The locator group consisted of 7 men and 14 women with a median age of 62 years (range, 44–80 years), and the bar group consisted of 10 men and 6 women with a median age of 58 years (range, 26–76 years). The protocol for the study was approved by the ethical review committee of Erciyes University, Kayseri, Turkey.

Only healthy individuals were included, and the medical history of each individual was checked for factors known to affect carriage of oral *Candida*, ie, cardiovascular diseases, diabetes mellitus, kidney problems, rheumatism, blood diseases, immunosuppression, and cancer therapy (radio- or chemotherapy). In addition, individuals who reported wearing their overdentures for at least 12 hours per day over the previous year and who had taken no antibiotic, antifungal, or steroid medication for at least the previous 3 months were included in the study.

Implants and prosthodontic rehabilitation

Standard Plus Straumann implants (Institut Straumann AG, Waldenburg, Switzerland) had been placed in 15 patients, MIS Seven implants (MIS Implants Technologies Ltd, Shlomi, Israel) in 8 patients, Swissplus implants (Zimmer Dental, San Diego, Calif) in 6 patients, and Osseotite implants (Biomet 3i, Palm Beach, Fla) in 8 patients. All implants had been placed according to a transgingival unloaded healing protocol and were loaded approximately 3 months after placement.

All dentures had been fabricated with a metallic skeleton and hot-curing acrylic resin. The dentures were attached to either locator attachments or fabricated Hader bars with Teflon clips. The female parts of the attachments were connected to the denture base with hot-curing acrylic resin during the laboratory process. During fabrication of the dentures, the laboratory technician left significantly more space in the denture base around bar clips than around the locator attachments owing to the nature of the attachment design (ie, to allow for freedom of prosthetic movement around the bar and implants during function).

Clinical assessment

All patients were examined by 1 periodontologist and 1 prosthodontist. They collected full medical and dental histories and conducted comprehensive oral examinations. The age and fit of dentures, together with existing denture hygiene practices, were recorded. In addition, plaque[26] and gingival[27] indices were measured on 4 sides (mesial, distal,

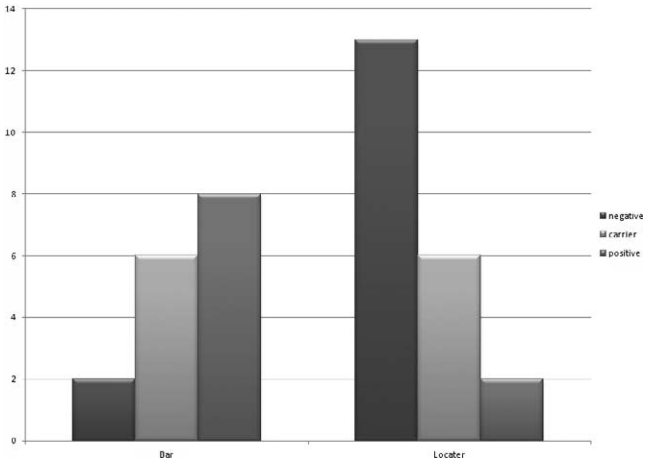

Figure 1. Distribution of cfu values of samples from inner denture surfaces.

buccal, lingual) of each implant and scored from 0–3, and the mean value was used in calculations. If present on the palatal mucosa, DRS was classified as Newton's type I (localized erythematous), type II (diffuse erythematous), or type III (hyperplastic granular). Patients with no evidence of DRS were classified as uninfected (score of 0). As a result, Newton's type I, II, and III patients were defined as having DRS, and Newton's type 0 patients were defined as lacking DRS in this study.

Mycological examination

For locator-retained overdentures, 4 samples were obtained surrounding the 2 locator attachments and from processing caps on the overdentures using sterile cotton swabs. For bar-retained overdentures, 4 samples were obtained from 2 bar attachments of each distal-placed implant and from the corresponding 2 clips on the overdentures, which were located in front of the bar attachments. To standardize the effect of diurnal variation, meals, and toothbrushing, the sampling was carried out at midmorning at least 2 hours after drinking, eating, or any oral hygiene procedure. During the sampling process, sterile cotton swabs were rotated twice at the surroundings of the attachments, processing caps, and bar clips. Each swab was then transferred into 1 mL of sterile phosphate-buffered saline and rinsed by vortexing vigorously for 30 seconds between dilutions to remove cells from the swab and to maximize the homogeneity of the suspensions. Each dilution (100 μL) was spread-plated onto Sabouraud dextrose agar (SDA; Life Technologies, Paisley, UK) and incubated at 25°C for 24–48 hours. Quantitative and qualitative microbiological assessments were performed, recording the total numbers of colonies (cfu) and their morphological characteristics. Yeast colonies were classified according to the number of cfu as follows: negative (0 cfu/mL), carrier ($<$400 cfu/mL), and positive ($\geq$400 cfu/mL).[28] Isolated yeasts were identified using standard microbiological procedures, which included identification based on germ-tube formation, colony morphology on SDA, and morphological characteristics on cornmeal agar, urea hydrolyses, and carbohydrate assimilation test using an API 20°C Aux yeast identification kit (BioMérieux, Marcy l'Etoil, France).[29]

Statistical analysis

Data are expressed as frequencies and percentages in cross-tabulation table formats. Pearson's chi-square tests and Fisher exact tests were used for comparisons. A value of $P < .05$ was considered statistically significant.

Results

Description and microbiological assessment

Of the 37 individuals with overdentures recruited for this study, 16 were using a bar attachment and 21 were using a locator attachment.

Eight and 2 patients (50% and 9.5%) using bar-retained and locator-retained overdentures, respectively, exhibited $\geq$400 cfu/mL isolated from the inner surfaces of dentures. Samples collected from inner denture surfaces yielded $<$400 cfu/mL in 6 (37.5%) individuals with bar-retained overdentures and 6 (28.6%) individuals with locator-retained overdentures. No yeast was isolated from the inner surfaces of bar-retained and locator-retained overdentures of 2 (12.5%) and 13 (61.9%) patients, respectively (Figure 1).

Eight (50%) and 0 (0%) patients using bar-retained and locator-retained overdentures exhibited $\geq$400 cfu/mL yeasts isolated from attachment surroundings. Seven (43.8%) and 10 (47.6%) patients with bar-retained and locator-retained overdentures showed $<$400 cfu/mL isolated from attachment surroundings. No yeast was isolated from the attachment surroundings of 1 (6.3%) and

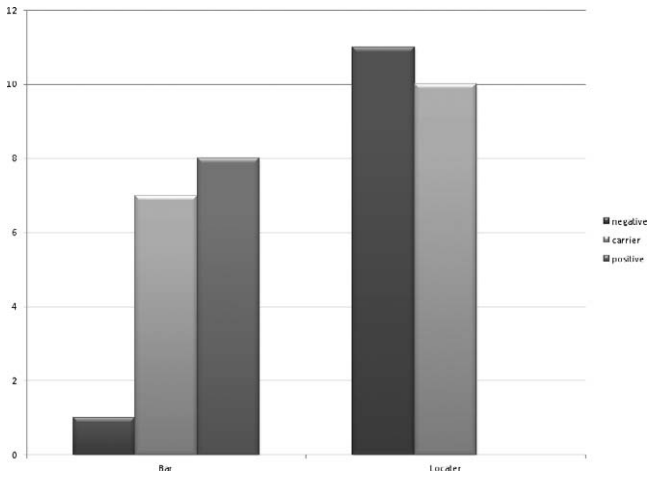

Figure 2. Distribution of cfu values of samples isolated from abutment surroundings.

11 (52.4%) patients with bar-retained and locator-retained overdentures (Figure 2).

Chi-square tests showed significant differences in the cfu isolated from the attachments and inner surfaces of locator- and bar-retained overdentures ($P < .05$).

C albicans was the most common species in both evaluations, isolated from 81.3% of patients using bar-retained overdentures and 38.1% of those using locator-retained overdentures. *C glabrata* was the second most common species, isolated from 37.5% of patients using bar-retained overdentures and 23.8% of those using locator-retained overdentures.

Candida kefyr and *Candida norvegensis* were isolated from 1 patient each using bar- and locator-retained overdentures. Mixed-species populations (*C albicans* and *C glabrata*) were isolated from 25% of patients using bar-retained overdentures and 19% of those using locator-retained overdentures.

Table 1

Distribution of *Candida* species on bar- and locator-retained overdentures of patients with denture-related stomatitis

Candida Species	Bar	Locator	*P*
No yeast	0	6 (40%)	*
C albicans	9 (56.3%)	4 (26.7%)	
C glabrata	3 (18.8%)	1 (6.7%)	
Mixed	4 (25%)	4 (26.7%)	

*$P < .05$.

Table 2

Distribution of locator- and bar-retained overdentures correleted with hygiene status ($P > .05$)

Hygiene Status	Bar	Locator
Smoking	2 (12.5%)	3 (14.3%)
No smoking	14 (87.5%)	18 (85.7%)
Overnight removal	8 (50%)	15 (71.4%)
No overnight removal	8 (50%)	6 (28.6%)
Gingival index		
0	0	3 (14.3%)
1	2 (12.5%)	6 (28.6%)
2	11 (68.8%)	6 (28.6%)
3	3 (18.8%)	6 (28.6%)
Plaque index		
0	0	3 (14.3%)
1	2 (12.5%)	6 (28.6%)
2	10 (62.5%)	6 (28.6%)
3	4 (25%)	6 (28.6%)

Clinical assessments

While DRS developed in all patients using bar-retained overdentures, it developed in only 71.4% of patients using locator-retained overdentures.

C albicans was detected in 56.3% of patients with DRS, *C glabrata* in 18.8% of these patients, and mixed species in 25% of patients with DRS who were using bar-retained overdentures.

C albicans was detected in 26.7% of patients with DRS, *C glabrata* in 6.7% of these patients, and mixed species in 26.7% of patients with DRS using locator-retained overdentures. A chi-square test found a significant difference between locator- and bar-retained overdentures according to *Candida* species in patients with DRS ($P < .05$; Table 1).

A chi-square analysis of *Candida* species found no significant difference between patients with and without DRS using locator-retained overdentures ($P > .05$). No patient without DRS used bar-retained overdentures.

Table 2 shows the distribution of locator- and bar-retained overdentures correlated with smoking habit, overnight removal, and plaque and gingival indices. A chi-square analysis found no significant difference in these parameters between locator- and bar-retained overdentures ($P > .05$).

Table 3 shows the distribution of patients with and without DRS who used locator- and bar-retained overdentures correlated with smoking habit, overnight removal, and plaque and gingival indices. A significant impact was found in a chi-square analysis of gingival and plaque indices of

Table 3

Distribution of patients with and without denture-related stomatitis (DRS) with locator- and bar-retained overdentures correlated with hygiene status

	Bar			Locator		
Hygiene Status	No DRS	DRS	*P*	No DRS	DRS	*P*
Smoking	0	2 (12.5%)		0	3 (14.3%)	
No smoking	0	14 (87.5%)		6 (28.6%)	12 (57.1%)	
Overnight removal	0	8 (50%)		5 (23.8%)	10 (47.6%)	
No overnight removal	0	8 (50%)		1 (4.8%)	5 (23.8%)	
Gingival index						
0	0	0		0	3 (14.3%)	*
1	0	2 (12.5%)		4 (19%)	2 (9.5%)	
2	0	11 (68.8%)		0	6 (28.6%)	
3	0	3 (18.8%)		2 (9.5%)	4 (19%)	
Plaque index						
0	0	0		0	3 (14.3%)	*
1	0	2 (12.5%)		4 (19%)	2 (9.5%)	
2	0	10 (62.5%)		0	6 (28.6%)	
3	0	4 (25%)		2 (9.5%)	4 (19%)	

*$P < .05$.

locator-retained overdentures in patients with and without DRS ($P < .05$). No patients without DRS used bar-retained overdentures; therefore, no statistical analysis could be performed.

Table 4 shows the distribution of cfu values of samples isolated from inner denture surfaces and abutment surroundings of maxillary and mandibular overdentures. Five patients (17.9%) using mandibular overdentures and 5 patients (55.6%) using maxillary overdentures exhibited ≥400 cfu/mL isolated from the inner surfaces of dentures. Samples collected from inner denture surfaces yielded <400 cfu/mL in 10 (35.7%) individuals with mandibular overdentures and 2 (22.2%) individuals with maxillary overdentures. No yeast was isolated from the inner surfaces of mandibular and maxillary overdentures of 13 (46.4%) and 2 (22.2%) patients, respectively.

Six (21.4%) and 2 (22.2%) patients using mandibular and maxillary overdentures, respectively, exhibited ≥400 cfu/mL yeasts isolated from attachment surroundings. Ten (35.7%) and 7 (77.8%) patients with mandibular and maxillary overdentures, respectively, showed <400 cfu/mL isolated from attachment surroundings. No yeast was isolated from the attachment surroundings of 12 (42.9%) and 0 (0%) patients with mandibular and maxillary overdentures, respectively.

Chi-square tests showed significant differences in the cfu values isolated from the attachment surfaces of mandibular and maxillary overdentures ($P < .05$). However, no significant difference was found in the cfu values isolated from the inner denture surfaces of mandibular and maxillary overdentures ($P > .05$).

Discussion

We evaluated the prevalence of DRS in locator- and bar-retained overdenture wearers and its association with particular colonizing *Candida* species. *Candida* species cfu values of bar-retained overdenture wearers were significantly higher than those of locator-retained overdenture wearers. While DRS developed in all patients using bar-retained overdentures, DRS developed in only

Table 4

Distribution of cfu values of samples isolated from inner denture surfaces and abutment surroundings of maxillary and mandibular overdentures

	Prosthesis Site		
	Mandibular	Maxillary	*P*
Inner denture surfaces			
Negative	13 (46.4%)	2 (22.2%)	
Carrier	10 (35.7%)	2 (22.2%)	
Positive	5 (17.9%)	5 (55.6%)	
Abutment surroundings			
Negative	12 (42.9%)	0 (0.0%)	*
Carrier	10 (35.7%)	7 (77.8%)	
Positive	6 (21.4%)	2 (22.2%)	

*$P < .05$.

71.4% of those using locator-retained overdentures. In this study, the data support the rejection of the null hypothesis that there would be no difference between locator- and bar-retained overdentures in terms of the prevalence of DRS and colonization by *Candida* species of the inner surfaces of dentures and attachment surrondings.

C albicans plays an important role in oral candidal infections.[30] The higher prevalence of *C albicans* is explained by its greater capability to adhere to mucosal surfaces, which is considered the first step in the pathogenesis of oral candidiasis.[31] *C albicans* was the most widespread species in the saliva, palatal mucosa, and tongue mucosa samples of the individuals who developed DRS, suggesting that this species is the major cause of prosthetic stomatitis or DRS development.[31] Whereas *C albicans* was the most common species isolated from the inner surfaces of overdentures and attachment surroundings in both groups using bar-retained overdentures (81%) and locator-retained overdentures (38%), the prevelance of *C albicans* was higher in those using bar-retained overdentures. This finding is similar to that reported by Dar-Odeh and Shehabi,[18] who found that *C albicans* was isolated from 73% of patients with DRS. In another study, denture wearers showed a 67% rate of *C albicans* colonization.[32] Also, the higher occurrence of *Candida* species in the abutment surroundings of maxillary overdentures could be related to the application of bar-retained overdentures mostly to the maxilla.

Although *C albicans* has been shown to be the predominant isolate, other species have also been identified. Our data support this observation; similar to previous reports,[18–20] *C glabrata* was the second most frequently isolated species from bar-retained overdentures (37.5%) and locator-retained overdentures (23.8%), although others found the second most prevalent isolate to be *C tropicalis*.[31] Furthermore, we identified cases of mixed colonization of bar-retained (25%) and locator-retained (19%) overdentures. Significantly more *Candida* species were isolated from the inner surfaces and attachment surroundings of bar-retained overdentures than from locator-retained overdentures.

Our data showed significant differences between the *Candida* species isolated from bar- and locator-retained overdentures of patients with DRS. Previous reports demonstrated that *C albicans* was responsible for about 54%–74% of DRS cases.[17,33–35] In this study, *Candida* species were isolated from all (100%) bar-retained overdentures and 6 of 15 (60%) locator-retained overdentures in patients with DRS.

Dental plaque is an important factor in stomatitis in patients who wear dentures; therefore, cleaning of dentures and plaque removal are important steps in the maintenance of good oral health. According to our study, plaque and gingival index parameters increased the possibility of DRS prevalence in those using locator-retained overdentures. No patients without DRS were included in the bar-retained group; therefore, no statistical analysis could be performed. Ambard et al[36] evaluated the cleansability of overdentures retained by direct extracoronal resilient attachments (ERAs) and overdentures supported by a Hader bar. They found that the ERA group rated the overdentures better than the bar group did when comparing the ease of performing oral hygiene, although the results were not statistically significant.[36] Our findings indicate that gingival and plaque index values of bar-retained overdentures were worse than those of locator-retained overdentures, but these differences were not statistically significant, in agreement with the report of Ambard et al.[36] The small population may be the reason for this result. The worse periodontal parameters for bar-retained overdentures may be associated with the fact that cleaning is more difficult for bar attachments than locator attachments.

Continuous denture wearing and smoking habit appear to facilitate DRS by increasing local injury and the time of mucosal exposure to denture plaque. Sadamori et al[37] stated that DRS occurred more frequently in edentulous patients wearing dentures during sleep. However, according our results, the relationships of overnight denture wearing and smoking habit to DRS were not statistically significant for locator- or bar-retained overdentures (Tables 2 and 3). Although our results showed no significant association between these parameters and DRS, these factors should be further investigated using greater numbers of patients to draw firm conclusions.

Conclusions

The cfu values of *Candida* species were higher for bar-retained than locator-retained overdentures. To

prevent DRS, we suggest the use of locator attachments, rather than bar attachments; the provision of instruction for regular denture and attachment surface hygiene; and patient motivation to maintain adequate oral hygiene. However, future studies using a larger population will be necessary to determine the association between *Candida* species and DRS in bar- and locator-retained overdenture users.

Abbreviations

cfu: number of colonies
DRS: denture-related stomatitis
ERA: extracoronal resilient attachment
SDA: Sabouraud dextrose agar

References

1. Nevalainen MJ, Narhi TO, Ainamo A. Oral mucosal lesions and oral hygiene habits in the home-living elderly. *J Oral Rehabil*. 1997;24:332–337.

2. Sherman RG, Prusinski L, Ravenel MC, Joralmon RA. Oral candidasis. *Quintessence Int*. 2002;33:521–532.

3. Guggenheimer J, Moore PA, Rossie K, et al. Insulin dependent diabetes mellitus and oral soft tissue pathologies. II. Prevalence and characteristics of *Candida* and candidal lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2000;89:570–576.

4. Sakki TK, Knuuttila ML, Läärä E , Anttila SS. The association of yeasts and denture stomatitis with behavioral and biologic factors. *Oral Surg Oral Med Oral Pathol Oral Radio Endod*. 1997;84:624–629.

5. Cannon RD, Chaffin WL. Oral colonization by *Candida albicans*. *Crit Rev Oral Biol Med*. 1999;10:359–383.

6. Bilhan H, Sulun T, Erkose G, et al. The role of *Candida albicans* hyphae and *Lactobacillus* in denture-related stomatitis. *Clin Oral Investig*. 2009;13:363–368.

7. Calcagno AM, Bignell E, Warn P, et al. *Candida glabrata* STE12 is required for wild-type levels of virulence and nitrogen starvation induced filamentation. *Mol Microbiol*. 2003;50:1309–1318.

8. Cannon RD, Holmes AR, Mason AB, Monk BC. Oral *Candida*: clearance, colonization, or candidiasis? *J Dent Res*. 1995;74:1152–1161.

9. Cross LJ, Bagg J, Wray D, Aitchison T. A comparison of fluconazole and itraconazole in the management of denture stomatitis: a pilot study. *J Dent*. 1998;26:657–664.

10. Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol*. 2008;23:377–383.

11. Redding SW, Kirkpatrick WR, Saville S, et al. Multiple patterns of resistance to fluconazole in *Candida glabrata* isolates from a patient with oropharyngeal candidiasis receiving head and neck radiation. *J Clin Microbiol*. 2003;41:619–622.

12. Ramage G, VandeWalle K, Lopez-Ribot JL, Wickes BL. The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. *FEMS Microbiol Lett*. 2002;214:95–100.

13. Bagg J, Sweeney MP, Lewis MA, et al. High prevalence of non-albicans yeasts and detection of anti-fungal resistance in the oral flora of patients with advanced cancer. *Palliat Med*. 2003;17:477–481.

14. Coleman D, Sullivan D, Harrington B, et al. Molecular and phenotypic analysis of *Candida dubliniensis*: a recently identified species linked with oral candidasis in HIV-infected and AIDS patients. *Oral Dis*. 1997;3:96–101.

15. Oksala E. Factors predisposing to oral yeast infections. *Acta Odontol Scand*. 1990;48:71–74.

16. Lyon JP, da Costa SC, Totti VM, Munhoz MF, de Resende MA. Predisposing conditions for *Candida* spp. carriage in the oral cavity of denture wearers and individuals with natural teeth. *Can J Microbiol*. 2006;52:462–467.

17. Figueiral MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C. Denture-related stomatitis: identification of aetiological and predisposing factors—a large cohort. *J Oral Rehabil*. 2007;34:448–455.

18. Dar-odeh NS, Shehabi AA. Oral candidiasis in patients with removable dentures. *Mycoses*. 2003;46:187–191.

19. Dagistan S, Aktas AE, Caglayan F, Ayyildiz A, Bilge M. Differential diagnosis of denture-induced stomatitis, *Candida*, and their variations in patients using complete denture: a clinical and mycological study. *Mycoses*. 2009;52:266–271.

20. Zomorodian K, Haghighi NN, Rajaee N, et al. Assessment of *Candida* species colonization and denture-related stomatitis in complete denture wearers. *Med Mycol*. 2011;49:208–211.

21. Awad MA, Lund JP, Shapiro SH, et al. Oral health status and treatment satisfaction with mandibular implant overdentures and conventional dentures: a randomized clinical trial in a senior population. *Int J Prosthodont*. 2003;16:390–396.

22. Lachmann S, Kimmerle-Müller E, Gehring K, et al. A comparison of implant-supported, bar- or ball-retained mandibular overdentures: a retrospective clinical, microbiologic, and immunologic study of 10 edentulous patients attending a recall visit. *Int J Prosthodont*. 2007;20:37–42.

23. Setz JM, Wright PS, Ferman AM. Effects of attachment type on the mobility of implant-stabilized overdentures—an in vitro study. *Int J Prosthodont*. 2000;13:494–499.

24. Gotfredsen K, Holm B, Sewerin I, et al. Marginal tissue response adjacent to Astra Dental Implants supporting overdentures in the mandible. *Clin Oral Implants Res*. 1993;4:83–89.

25. Behneke A, Behneke N, d'Hoedt B. A 5-year longitudinal study of the clinical effectiveness of ITI solid-screw implants in the treatment of mandibular edentulism. *Int J Oral Maxillofac Implants*. 2002;17:799–810.

26. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121–135.

27. Loe H, Silness J. Periodontal disease in pregnancy. *Acta Odontol Scand*. 1963;21:533–551.

28. Abaci O, Haliki-Uztan A, Ozturk B, Toksavul S, Ulusoy M, Boyacioglu H. Determining *Candida* spp. incidence in denture wearers. *Mycopathologia*. 2010;169:365–372.

29. Larone DH. *Medically Important Fungi: A Guide to Identification*. Washington, DC: ASM Press; 1993.

30. Baena-Monroy T, Moreno-Maldonado V, Franco-Martinez F, Aldape-Barrios B, Quindos G, Sanchez-Vargas LO. *Candida albicans*, *Staphylococcus aureus* and *Streptococcus mutans* colonization in patients wearing dental prosthesis. *Med Oral Patol Oral Cir Bucal*. 2005;10:27–39.

31. McIntyre GT. Oral candidasis. *Dent Update*. 2001;28:132–139.

32. Daniluk T, Tokajuk G, Stokowska W, et al. Occurrence rate of oral *Candida albicans* in denture wearer patients. *Adv Med Sci*. 2006:51:77–80.

33. Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits,

denture cleanliness, presence of yeasts and stomatitis in elderly people. *J Oral Rehabil*. 2002;29:300–304.

34. Tavakol P, Emdadi S. Evaluation of prevalence of oral candidiasis in patients using complete denture wears. *Tehran Uni Med J*. 2001;59:86–90.

35. Vanden Abbeele A, De Meel H, Ahariz M, Perraudin JP, Beyer I, Courtois P. Denture contamination by yeast in elderly. *Gerodontology*. 2008;25:222–228.

36. Ambard AJ, Fanchiang JC, Mueninghoff L, Dasanayake AP. Cleansability of and patients' satisfaction with implant-retained overdentures: a retrospective comparison of two attachment methods. *J Am Dent Assoc*. 2002;133:1237–1242.

37. Sadamori S, Kotani H, Nikawa H, Hamada T. Clinical survey on denture stomatitis: the relation between the maintenance of denture and denture stomatitis. *Nippon Hotetsu Shika Gakkai Zasshi*. 1990;34:202–207.