Oral fungal colonization and oral candidiasis in renal transplant patients: The relationship to *Miswak* use

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Objectives. The aim of this study was to determine and compare the prevalence of oral candidal colonization and oral candidiasis in a group of medically stable renal transplant patients (RTPs) and age and sex-matched healthy control (HC) subjects.
Study design. The oral cavities of 58 RTPs and 52 HC subjects were clinically examined for the presence of oral candidiasis. Oral fungal colonization was determined by using the concentrated oral rinse technique.
Results. Prevalence of oral fungal colonization was not significantly higher in RTPs than in HC subjects (74.1% vs 59.6%, respectively; $P = .1$), but the density of growth was significantly higher in RTPs ($P < .0017$). Oral candidiasis was diagnosed in 15.5% of RTPs but in none of HC subjects ($P = .002$). RTPs who used a chewing stick (*Miswak: Salvadora persica*) for oral hygiene had a significantly lower prevalence of oral candidiasis ($P = .04$) compared with other RTPs.
Conclusions. RTPs are at high risk of developing oral candidiasis. More clinical investigations are needed to determine the antimycotic effect of *Miswak*. Regular oral screening is recommended for RTPs.
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Renal transplantation has evolved as a treatment option for patients with end-stage renal disease. Significant progress has been achieved during the past 2 decades in grafting and patient survival after renal transplantation. This is in part because of improvements in surgical and tissue-matching techniques, in addition to advances in antirejection drug therapy.[1] Renal transplant patients (RTPs) are usually conditioned with immunosuppressive agents such as corticosteroids, cyclosporin, azathioprine, and antilymphocyte monoclonal antibodies.[1,2] These agents are known to reduce the immune response of these patients— and consequently increase the risk of developing infections.[2] Among the different types of severe infections that may occur in RTPs, those caused by fungi are associated with the highest mortality rate, despite having a lower incidence than bacterial or viral infections. It has been estimated about 6% of patients in third-world countries develop systemic fungal infection after renal transplantation.[3] The skin of renal transplant recipients is considered to be more susceptible to fungal infection. One study showed that 42.5% of the patients were affected by dermatomycosis.[4] Esophageal candidiasis was diagnosed in 10.5% of RTPs in another study.[5]

Because of the chronic and extensive immunosuppressive therapy, organ transplant patients generally are expected to have a higher prevalence of oral candidal carriage and to be more susceptible to oral candidiasis than healthy subjects. However, to the best of our knowledge, this hypothesis has never been verified in RTPs. Only a few studies[6] have reported on the prevalence of oral candidiasis in RTPs. It seemed that those patients treated with a combination of cyclosporin, azathioprine, and prednisolone had a significantly higher prevalence of candidiasis than those taking either azathioprine and prednisolone or cyclosporin and prednisolone.[5]

The use of a chewing stick (*Miswak*: *Salvadora persica*) as an oral hygiene device is common in the Middle East and Africa. Recent in vitro studies have shown an antimycotic effect for an aqueous extract of *Miswak*.[7] Whether *Miswak* use has an effect on oral candidal carriage and infection in RTPs has not been tested.

The aims of this study were to determine the prevalence of oral fungal colonization and oral candidiasis in RTPs compared with that found in age-matched, sex-matched, and dental status–matched healthy control subjects and to identify possible risk factors. The study

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also aimed to investigate whether the use of *Miswak* as an oral hygiene device has an effect on oral candidal colonization or infection in RTPs and HC subjects.

MATERIAL AND METHODS

Patients

RTPs were recruited from the outpatient Renal Transplant Clinic of the King Fahad National Guard Hospital (Riyadh, Saudi Arabia). Only medically stable patients who had had a successful kidney transplant for at least 6 months were included in the study. The healthy control (HC) subjects consisted of apparently healthy age- and sex-matched individuals who were recruited from the outpatient clinic at the College of Dentistry, King Saud University (Riyadh, Saudi Arabia). All control subjects were attending for routine dental treatment, and none were seeking treatment for any oral mucosal disorder. Control subjects were excluded from the study if they had received antibiotic or corticosteroid therapy or antiseptic mouthwash during the past 3 months. Subjects who were diabetic, anemic, pregnant, or had any known disease or condition that predisposed to oral candidiasis were also excluded. To avoid the effect of removable dental prosthesis on oral candidal flora,[8] only dentate subjects not using any removable denture of any type were selected to participate in the study. Eight HC subjects were found to be wearing removable dentures and were not included in the study, whereas none of the examined RTPs were wearing a removable prosthesis. The oral cavities of all subjects were examined clinically under the light of the dental unit by one investigator (M.A.A.-M.). All findings including medical history, medications, and oral hygiene and smoking habits, and the results of these investigations were recorded.

A swab and smear were obtained from oral lesions suggestive of candidiasis. Cases of oral candidiasis were diagnosed clinically and confirmed by the demonstration of candidal hyphae or blastospores in stained smears and candidal colonies on culture plates.

Hematologic studies were performed only for RTPs and consisted of those requested routinely by the treating physician, including tests for blood glucose, blood urea nitrogen, serum creatinine, and serum cyclosporin level. The experimental procedures were explained for each subject, and those willing to participate in the study were asked to sign an informed consent form approved by the local ethics committee. All clinical examination and sampling took place between 8 AM and noon.

Microbiologic investigations

Oral mycologic flora was sampled by using the concentrated oral rinse technique described by Samaranayake et al.[9] Briefly, each subject was supplied with a universal container (Bippy Sterilin Ltd, Manchester, United Kingdom) containing 10 mL of sterile normal saline and requested to rinse the mouth thoroughly for a full 60 seconds, and then to return the rinse into the container. The material was centrifuged at 3500 rpm for 10 minutes (Labofuge GL; Heraeus Septech GMBH, Postlach, Germany). The supernatant was discarded, and the precipitate was mixed with 1 mL of sterile saline and agitated for 30 seconds on a vortex mixer (Touch plate mixer, LAB Line Instruments Inc, Rose Park, Ill) for appropriate disaggregation of the microorganisms. Subsequently, 0.1 mL of the rinse was inoculated on a culture plate containing Sabouraud's agar (Oxoid Ltd, Basingstoke, Hampshire, England). The swabs obtained from suspected lesions of candidiasis were streaked on similar plates, and all culture plates were incubated aerobically for 48 hours at 37°C. Smears were gram-stained and examined microscopically under ×40 magnification for the presence of candidal hyphae.

Microbiologic identification and quantification

Candidal growth was identified as round, smooth, creamy white colonies and was confirmed microscopically by observing the blastospore phase in a direct wet mount film under ×40 magnification. The number of colonies on rinse culture plates was counted and multiplied by 10 and expressed in colony-forming units per milliliter of rinse (CFU/mL). Culture plates that were negative for candidal growth were incubated at room temperature for an additional 72 hours and then re-examined before being discarded as negative. *Candida* species were identified by germ tube formation[10] and by the yeast identification system API 20C AUX[11] (bioMérieux, Marcy l'Etoile, France). *Aspergillus* species were identified macroscopically according to the colony morphology, color, and wet-mount preparation.[12]

Statistical analysis

Data of RTPs and HC subjects were analyzed by using the Pearson chi-square test or the Fisher exact test for categorical variables. The Mann-Whitney *U* test and the Student *t* test were used to determine differences among continuous variables. The 1-way ANOVA test was used to compare the mean colony-forming units per milliliter among various age groups, and the Pearson test was used to correlate mean colony-forming units per milliliter with blood investigation results. Data were analyzed by using the statistical analysis package SPSS, version 9.0 (SPSS Inc, Chicago, Ill). P values $< .05$ were considered to be significant.

RESULTS

A total of 58 RTPs (22 women and 36 men) and 52 HC subjects (18 women and 34 men) participated in the study. The mean age of RTPs was 39.2 years (SD, 12.8), with a range of 16 to 62 years. The mean age of HC subjects was 37.1 years (SD, 11.6), with a range of 16 to 60 years. The mean duration of renal transplant was 51.6 months (SD, 31.9), ranging from 7 to 125 months. A total of 39 (67.2%) RTPs had a cadaver renal transplant, whereas living unrelated donors and living relatives were used for transplantation in 10 (17.2%) and 9 (15.5%) of the RTPs, respectively. Table I presents the medications used by the RTPs.

Table I. Medications used by RTPs

Medications used	*No. of RTPs (%)*
Cyclosporin	56 (96.6)
Prednisone	58 (100)
Azathioprine	17 (29.3)
Mycophenolate mofetil	3 (5.2)
Tacrolimus	1 (1.7)
Calcium channel blockers	37 (63.8)
Other antihypertensive agents	37 (63.8)

RTPs, Renal transplant patients.

Oral fungal flora

Asymptomatic oral fungal colonization was detected in 43 RTPs (74.1%) with 3002 mean CFU/ml (SD, 4548) and in 31 HC subjects (59.6%) with 656 mean CFU/ml (SD 1732). Although the prevalence of oral fungal colonization was not statistically different between the 2 groups ($P = .1$), the density of fungal growth was significantly higher in RTP ($P < .0017$). The density and prevalence of oral fungal colonization in RTPs and HC subjects were not influenced by age, sex, smoking and oral hygiene, or dental status.

Although the prevalence of oral fungi was lower in RTPs using *Miswak* (64%) than in those not using *Miswak* (81.8%), the difference was not statistically significant (Table II; $P = .13$). In contrast, the prevalence of oral fungal colonization was significantly lower in HC subjects using *Miswak* than in those not using *Miswak* (31.2% vs 72.2%, respectively; $P = .002$; Table II). The prevalence and density of oral fungal colonization in RTPs were not correlated with the duration of transplant survival ($P = .23$ and .6, respectively).

The fungal species isolated from the oral cavity of the study population free of oral candidiasis are presented in Table III. *Candida albicans* was the most frequently isolated species from RTPs and HC subjects.

Oral candidiasis

Among the RTPs, 9 (15.5%) showed clinical and microbiologic evidence of oral candidiasis. All 9 patients had erythematous candidiasis, although 1 of those had both erythematous candidiasis and angular cheilitis. During the patient interview, only 4 of RTPs with erythematous candidiasis reported a mild burning sensation on the dorsum of the tongue. Seven cases of erythematous candidiasis were located on the dorsum of the tongue, whereas the remaining 2 cases were located on both the dorsum of the tongue and on the hard palate. *C albicans* was isolated from swabs of 5 patients, whereas *Candida famata* and *Candida dubliniensis* were isolated from 2 patients. None of the HC subjects had oral candidiasis ($P = .002$).

Statistical analysis showed no difference in prevalence of oral candidiasis between smokers and nonsmokers ($P = .63$) or between those who regularly brushed their teeth and nonbrushing patients ($P = .7$). Similarly, oral candidiasis was not related to the patient's age, sex, or duration of transplantation. Although the mean level of plasma cyclosporin was higher in RTPs with oral candidiasis than in other RTPs, the difference was not statistically significant. However, 8 of 33 (24.2%) RTPs not using *Miswak* had oral candidiasis, which was significantly higher than 1/25 (4%) of those using *Miswak* ($P = .04$).

DISCUSSION

Candida species are normal oral commensals, and the transition of these innocuous commensals to pathogenic organisms may be associated with the virulence attributes of the organism. Nevertheless, it is generally accepted that the host factors are of critical importance in the development of the disease state.[13] In addition, it is widely accepted that most of the clinical oral candidal infections are endogenous—rather than exogenous—in origin.[14] Historically, a number of synonyms such as *colonization, carriage*, and *infestation* have been used in the literature to indicate the intraoral presence of yeast as commensals in the absence of clinically evident infection. The term *colonization* was used preferentially here to indicate the presence of yeast in the absence of infection. The term *infection* was used to indicate clinically evident fungal disease. The present investigation provides initial data on the prevalence and density of oral fungal colonization in RTPs in comparison with those of HC subjects.

The present investigation showed that the oral cavity of 74.1% of renal transplant recipients was colonized with fungi, which was higher than in sex- and age-matched HC subjects (59.6%), although the difference was not statistically significant. This may be explained by the fact that our patients were all medically stable cases receiving a maintenance dose of immunosuppressants. Our finding in RTP was very close to the

Table II. Oral fungal colonization among subgroups of the study subjects

Subgroup	*No. of RTPs*	*No. (%) of RTPs, fungal carriers*	*HC subjects*	*No. (%) of HC subjects, fungal carriers*
Men	36	27 (75)	34	18 (52.9)
Women	22	16 (72.7)	18	13 (72.2)
Miswak user	25	16 (64.0)	16	5 (31.2)
Non-*Miswak* user	33	27 (81.8)	36	26 (72.2)

HC, Healthy control.

Table III. Fungal species isolated from the oral cavities of noninfected RTPs and HC subjects

Species	*RTPs (n = 43*)*	*HC subjects (n = 31)*
Candida albicans	22 (51.2%)	23 (74.2%)
Cryptococcus humicolus	15 (34.9%)	2 (8.7%)
Candida famata	3 (6.9%)	0 (0%)
Candida guilliermondii	1 (2.3%)	2 (6.5%)
Candida krusei	1 (2.3%)	0 (0%)
Candida parapsilosis	0 (0%)	1 (3.2%)
Aspergillus fumigatus	1 (2.3%)	2 (6.5%)
Candida tropicalis	1 (2.3%)	1 (3.2%)
Trichosporon mucoides	1 (2.3%)	0 (0%)

*Two RTPs had more than 1 species isolated.

75% oral fungal colonization reported in human immunodeficiency virus (HIV)–seropositive patients.[15] Apparently, immunosuppressive medications increase the predisposition to oral fungal colonization in RTPs.[5] The high number of fungal colony-forming units in RTPs compared with control subjects is consistent with that reported in HIV-positive patients.[15,16]

The finding that *C albicans* was the most commonly isolated species from both RTPs and HC subjects was expected, because it is frequently reported that this *Candida* species is the most commonly isolated in health and disease.[8,14] Non-*albicans* species were isolated more frequently from RTPs than from HC subjects, which may in part be attributed to the immunosuppressed condition of RTPs. Several studies have reported an increased prevalence of infections in immunocompromised patients caused by non-*albicans* species such as *Candida krusei, Candida tropicalis,* and *Candida glabrata.*[17,18]

Interestingly, some fungal species other than *Candida,* such as *Aspergillus, Cryptococcus*, and *Trichosporon* species, were detected among subjects of this study. *Aspergillus* species are widely distributed opportunistic fungal pathogens, the most common being *Aspergillus fumigatus.*[19] The inhalation of its airborne-disseminated spores can lead to the development of invasive and disseminated aspergillosis, especially in immunocompromised patients such as renal transplant recipients.[20,21] The administration of a high dose of corticosteroids poses a significant risk for invasive infection with *Aspergillus* species in RTPs.[22] *Aspergillus* and *Cryptococcus* species have been documented as causative of infection of the central nervous system in RTPs.[23] *Trichosporon mucoides* is one of the most common strains of fungi that can cause disseminated trichosporonosis, a severe and lethal opportunistic infection in immunosuppressed organ transplant patients.[24] The significance of isolation of these species from RTPs without a clinical infection is difficult to interpret but necessitates careful follow-up.

Miswak is a stick usually derived from either the branches or stem of a bush known in the Middle East as "Arak" *(Salvadora persica)* and cut into pieces 10- to 25-cm long with a 2-cm diameter. *Miswak* is commonly used for brushing teeth and religious purposes in the Middle East, including Saudi Arabia and Gulf States, and parts of Asia and Africa. As an oral hygiene device, *Miswak* is usually used 3 to 10 times daily.[25] Many studies have demonstrated the antibacterial, anticaries, antiperiodontopathic, and antifungal properties of the contents of *Miswak.*[7,26] Both RTPs and HC subjects using *Miswak* as part of their daily oral hygiene practice showed lower levels of oral candidal carriage than did those not using *Miswak.* However, the difference was significant only in the HC group. Similarly, a significantly lower prevalence of oral candidiasis was encountered among RTPs using *Miswak* in their oral hygiene practice. This observation is in agreement with the results of in vitro studies demonstrating an antimycotic effect of an aqueous extract of *Miswak* at different concentrations.[7,26] This effect was probably caused by one or more of the root contents of *Miswak,* which include chlorine, trimethylamine, alkaloid resin, and sulfur compounds.[7]

Data on the prevalence of oral candidiasis in RTPs are very sparse. A literature review revealed only 1 study on the prevalence of oral candidiasis in these patients.[6] Oral candidiasis in immunosuppressed patients such as those who are HIV-seropositive is prevalent, and the majority of the infections were almost equally divided between erythematous and pseudomembranous candidiasis.[27] Wide variations were reported on the prevalence of oral candidiasis in these patients because of differences in the levels of immunosuppression, diagnostic criteria, and studied

populations.[28] The 15.5% prevalence of oral candidiasis among RTPs in the present investigation is of significance because none of the control subjects had oral candidal infection. This prevalence is also higher than the 10.1% prevalence of oral candidiasis reported by King et al[6] and the 10.5% prevalence of esophageal candidiasis reported by Gupta et al[5] in RTPs. In the latter study, 43% of those with esophageal candidiasis also had pseudomembranous candidiasis. However, this prevalence rate is lower than that found in patients with HIV/acquired immune deficiency syndrome (AIDS), 30% to 60% of whom have oral candidiasis.[28] The difference between RTPs and patients with HIV/AIDS may be related to the differences in the level of immunosuppression. RTPs commonly have candidiasis develop when they first receive their transplant while taking an initial high dose of immunosuppressant therapy to prevent organ rejection.[2] Our RTPs were all medically stable patients who had a successful transplant for at least 6 months, and were receiving a maintenance dose of immunosuppressants.

All cases of oral candidiasis among our RTPs were of the erythematous type. King et al[6] observed that 3.8% of RTPs have erythematous candidiasis, the type that is strongly associated with HIV-positive immunosuppressed patients. Our results support the observation that erythematous candidiasis is not only restricted to patients with HIV/AIDS, but other immunosuppressed patients may have this type of candidal infection.[6]

Oral *Candida* can cause significant problems in RTPs. Fatal *Candida* esophagitis has been reported in 2 RTPs with diabetes.[29] In a subsequent prospective study, *Candida* esophagitis was diagnosed in 2.2% of the study population, and all infected patients had diabetes mellitus.[30] Diabetes mellitus is known to predispose to oral candidal infection,[31] and hence forms an additional risk factor for the development of disseminated candidiasis in immunosuppressed RTPs. The high prevalence of oral candidal colonization and clinical candidiasis in RTPs indicates the importance of regular and careful oral screening in these patients.

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