Oral Microflora in Patients on Hemodialysis and Kidney Transplant Recipients

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Introduction. We aimed to determine oral microflora of patients on long-term hemodialysis and kidney transplant recipients, and to compare them with individuals without kidney disease.
Materials and Methods. We studied on 3 groups including patients on at least 6 months of hemodialysis, kidney transplant recipients for more than 2 years, and controls with a normal kidney function. Staining and culture were applied for samples from the dorsum of the tongue and the oral floor in order to detect aerobic and anaerobic bacteria and *Candida*.
Results. The participants were 49 patients on hemodialysis, 50 kidney transplant recipients, and 50 volunteers in the control group. The abundance of *Candida* was significantly higher in the hemodialysis and transplant groups compared with the control group. The mean of various microorganisms was found to be significantly higher in the hemodialysis group than the control group (P = .03); however, the frequency of these microorganisms in the transplant group was lower than that in the hemodialysis group. Adjusting for confounding factors, the odds of having *Candida* in the hemodialysis and transplant groups were 3.54 (95% CI, 1.21 to 10.41) and 3.49 (95% CI, 1.27 to 9.18) times higher compared to the control group, respectively.
Conclusions. Hemodialysis and kidney transplantation could affect oral microflora. *Candida* was significantly more frequent in these patients compared to healthy adults. *Streptococcus mutans*, *Lactobacilli*, *Porphyromonas*, and *Candida* is seen slightly less frequently after kidney transplantation, which might be in favor of promising effects of kidney transplantation on oral microflora.

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INTRODUCTION

There is an about 5% annually increase in the number of patients with chronic kidney failure, patients undergoing hemodialysis or kidney transplantation.[1] Thereby, there is a higher chance to see such patients in a dental office. In the literature, there are controversies regarding the oral health status of these patients; some studies report a high prevalence of dental caries, periodontal diseases, and related bacteria.[2-6] They suggest the risk of cariogenicity and the prevalence of cariogenic bacteria are high in these patients. In some studies, there were not any differences in the gingival index, plaque index, probing depths, and periodontal bacteria in these patients in comparison to the general population.[1,7,8] Others believe uremia and elevated pH state in the mouth decrease the risk of cariogenicity and amount of cariogenic bacteria

among these patients. The purpose of this study was to evaluate the oral microflora in patients on hemodialysis and kidney transplant recipients compared with a control group of individuals without kidney diseases. We also aimed to clarify the possible role of kidney transplantation on oral microflora.

MATERIALS AND METHODS

Patients

This study was conducted in 2 university hospitals in Tehran, Iran. It was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. Participants were included in this investigation after providing written consent.

The sample size was determined to be 149 according to the sample size calculation and previous studies in the literature. The participants consisted of 3 groups: (1) patients with end-stage renal disease who were on hemodialysis for at least 6 months, (2) patients who had successfully undergone kidney transplantation at least 24 months prior to the study, and (3) controls, consisting of volunteers without any obvious systemic diseases. Normal kidney function was verified by measurement of blood urea nitrogen and serum creatinine level in this group. The exclusion criteria were periodontal treatment within the previous year; taking antibiotics and using mouthwash during a week prior to the oral exploration; being seropositive for the human immunodeficiency virus, hepatitis C virus, or hepatitis B virus; and history of maxillofacial trauma.

Information regarding the age, gender, hemodialysis duration, date of kidney transplantation, biochemical parameters, daily medications, systemic diseases, and periodontal examination (plaque index) were obtained from all patients and controls.

Oral and Dental Examinations

Oral and dental clinical examinations were carried out in an equipped dental unit of the hospitals. Oral health status was measured using the *plaque index*. Plaque index is a criterion for evaluation of oral health status that comes from dividing the number of plaques covering teeth surfaces to the total number of teeth surfaces. We can see the surfaces covered with plaque by using a disclosing tablet that clearly shows the plaque by changing the color of tooth surface into pink.

Staining and Culture

Samples were taken using an alginate swab in the same systematic manner as follows: the swab was rotated to remove saliva from the dorsum of the tongue and the oral floor. The swab was immediately placed into a sterile bottle containing 3 mL of Calgon-Ringer's solution to maintain the viability of microorganisms collected and stored on ice. Within 2 hours of collection, the swabs were transported using an ice dish to the Oral Microbiology Laboratory for processing.

Each sample was processed as 2 separate parts. Ten-fold serial dilutions were prepared in Fastidious Anaerobic Broth and 100 µL aliquots of the appropriate dilutions were inoculated onto selective and nonselective media. Mitis Salivarius Agar (MSA) supplemented with 0.1% potassium tellurite was prepared for the total streptococcal count. The plates were incubated for 7 days in an anaerobic chamber at 37°C. For the enumeration of *Streptococcus mutans*, MSA supplemented with 0.1% potassium tellurite, bacitracin 0.2 U/mL, and sucrose 15% w/v (BMSA) was prepared. The plates were anaerobically incubated for 3 days at 37°C. Rogosa agar was prepared for the enumeration of *Lactobacillus* species and anaerobically incubated at 37°C for 7 days. For the enumeration of *Candida* species, Sabouraud Dextrose Agar was used. Potassium hydroxide staining and culture were used to evaluate the presence of *Candida*. The plates were aerobically incubated for 2 days at 37°C. For total aerobic and anaerobic counts, Fastidious Anaerobic Agar supplemented with 5% (v/v) defibrinated horse blood was used. The plates for aerobic counts were incubated at 37°C for 3 days. The plates for anaerobic counts were incubated at 37°C in an anaerobic chamber with an atmosphere consisting of 90% nitrogen, 5% hydrogen, and 5% carbon dioxide, for 7 days.

Gram staining was the primary step for identification of *S mutans*, where bacteria appeared as gram-positive cocci. Then, from the BMSA plates, 2 colonies were picked and inoculated into separate 3-mL volumes of Todd Hewitt Broth and aerobically incubated for 24 hours. Gram staining was performed for each broth. Colonial morphology and Gram staining was used for identification

of *Lactobacillus* species. The microbiologist who performed all cultures and laboratory evaluation was blinded to grouping of the samples.

Statistical Analyses

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were described as mean ± standard deviation, and categorical variables were shown as 95% confidence interval (95% CI) and frequency (percent). Bivariate associations between the studied variables were analyzed with the Student *t* test, chi-square test, Pearson correlation test, 1-way analysis of variance, and repeated measures, where appropriate. Linear and logistic regressions were used to eliminate confounding factors. Post hoc and Benferroni tests were performed to evaluate any differences among the groups. A *P* value less than .05 was considered significant.

RESULTS

A total of 149 individuals were evaluated (63 men and 86 women). There were 49 and 50 patients in the hemodialysis and transplant groups, respectively, and 50 healthy adults in the control group. Gender distribution, blood urea nitrogen and serum creatinine levels, and the plaque index (to obviate confounding effect of oral hygiene) were not significantly different between the three groups (Table 1).

Table 2 shows isolation frequency, abundance of *S mutans*, *Lactobacilli*, *Porphyromonas*, and *Candida* after logarithmic conversion in all groups. As it shows, the abundance of *Candida* was significantly higher in the patients of the hemodialysis and transplant groups compared with the control group. The abundance of other microorganisms was not significantly different between the three groups.

Repeated measures test showed a significant difference in the mean of various microorganisms among all groups (*P* = .03). This difference was found significant between the hemodialysis and the control groups (*P* = .03; Post hoc test; Benferroni method). Linear regression analysis showed that isolation abundance of *Candida* was significantly

Table 1. Characteristics of Patients on Hemodialysis, Kidney Transplant Recipients, and controls*

Characteristic	Hemodialysis	Transplant	Control	*P*
Age	55.8 ± 14.4†	40.1 ± 15.1	46.3 ± 12.7	< .001‡
Gender				
Female	27 (55.1)	26 (52.0)	33 (66.0)	
Male	22 (44.9)	24 (48.0)	17 (34.0)	.33
Creatinine, mg/dL	…	1.42 ± 0.50	0.99 ± 0.16	< .001
Blood urea nitrogen, mg/dL	…	20.5 ± 9.8	16.6 ± 5.1	.16
Plaque index	80.8 ± 5.9	77.4 ± 9.2	78.5 ± 6.7	.15‡
Systemic Diseases				
Hypertension	20 (40.8)	12 (24.0)	5 (10.0)	.002
Diabetes mellitus	10 (20.4)	2 (4.0)	3 (6.0)	.01
Cardiovascular disease	8 (16.3)	2 (4.0)	0	.002
Others	2 (4.1)	1 (2.0)	7 (14.0)	.07
Denture wear	16 (32.7)	4 (8.0)	4 (8.0)	.001

*Values are mean standard deviation for quantitative variables and frequency (percent) for qualitative ones. Ellipses indicate not included in the analysis.
†It was significantly higher compared to both transplant and control groups, based on the Bonferroni test.
‡Based on the 1-way analysis of variance.

Table 2. Isolation Frequency and Abundance (Log_{10} CFU/mL) of Oral Microbial Flora*

	Hemodialysis		Transplant		Control		*P*	
Oral Microbial Flora	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency	Abundance†	Frequency
Streptococcus mutans	3.55 ± 0.73	48 (98.0)	3.40 ± 1.05	47 (94.0)	3.70 ± 0.54	50 (100)	.15	.23
Lactobacilli	0.49 ± 1.08	9 (18.4)	0.42 ± 1.12	7 (14.0)	0.20 ± 0.70	4 (8.0)	.53	.32
Porphyromonas	0.31 ± 0.94	5 (10.2)	0.24 ± 0.98	3 (6.0)	0.20 ± 0.80	3 (6.0)	.71	.68
Candida	1.29 ± 1.63	19 (38.8)	1.26 ± 1.70	18 (32.0)	0.52 ± 1.09‡	9 (18.0)	.02	.05

*Values are mean standard deviation for quantitative variables and frequency (percent) for qualitative ones.
†Based on the 1-way analysis of variance.
‡It was significantly lower compared to both transplant and hemodialysis groups, based on the Bonferroni test.

lower in the control group compared to the hemodialysis and transplant groups (P = .04 and P = .01, respectively). Adjusting for all confounding factors, logistic regression revealed that the odds of having *Candida* in the hemodialysis and transplant groups were 3.54 and 3.49 times higher than that in the control group, respectively (95% CI, 1.21 to 10.41 and 1.27 to 9.18; respectively).

There was no relation between the duration of hemodialysis or transplant and microflora count (P = .06 and P = .60, respectively). There was no relation, either, between cariogenic bacteria (P = .60). Denture use had no effect on the count of *Candida* (P = .30). Hypertension, diabetes mellitus, and cardiovascular diseases had no significant relation with any of the microorganisms.

DISCUSSION

We performed a case-control study to determine any association of hemodialysis and kidney transplantation with oral microbial flora. Our results showed that the count of *Candida*, *Lactobacilli*, and *Porphyromonas* are higher in patients on hemodialysis and kidney transplant recipients compared with healthy individuals; however, only *Candida* was significantly more abundant. Comparing the former two groups together, the abundance of these microorganisms in kidney transplant patients was lower than those in the hemodialysis group.

Some previous studies reported a higher prevalence of *Candida* among the oral flora of patients on hemodialysis and kidney transplant recipients.[1,6] Results of our study are in accordance with these reports. Vesterinen and associates[1] noticed a higher oral incidence of *Candida* in patients with renal disease compared to controls, and Takeuchi and coworkers[6] reported a higher incidence of *Candida* in patients on hemodialysis compared to controls.

Some authors have attributed the higher oral count of *Candida* in patients on hemodialysis to the higher rate of denture use, administration of several drugs, and drug-induced xerostomia.[9-12] We did not find any relationship between *Candida* colony count and denture use. The results of the study by Takeuchi and coworkers[6] were in line with our findings in terms of the denture role. In transplant group, immunosuppressive drugs and long-term corticosteroid therapy make the patients immunocompromised and susceptible to opportunistic infections such as *Candida*. Haag-Weber and colleagues[13] reported cellular immunodeficiency in patients with chronic kidney failure and uremic state.

There are controversies about caries and cariogenic bacteria in patients with chronic kidney failure. In the literature, some authors have reported higher caries rate in these patients.[2-6] They suggest dental complications such as enamel hypoplasia and drug-induced xerostomia are quite common among them.[9-12] On the other hand, some other authors believe that uremic state and the higher oral pH decrease the risk of caries formation.[8,14,15] In our study, *S mutans* was higher in the control group compared with the two other groups and *Lactobacilli* count was lower in the control group than that in the two other groups. However, the differences were not statistically significant. Ertugrul and coworkers[16] noticed a lower incidence of *S mutans* in patients on hemodialysis compared to controls.

As previous reports have mentioned,[6,14] antibiotics and mouthwash may change oral microflora. Hence, in this study, all of the individuals who used these agents during a week before the evaluation were excluded. Since socioeconomic status may affect the oral hygiene, we selected our patients from two referral hospitals; both affiliated to Shahid Beheshti University of Medical Sciences (both located in North of Tehran), to reduce the likelihood of socioeconomic disparity. Our results showed that there was a significant difference in the mean of various microorganisms among all groups. This difference was found significant between the hemodialysis and the control groups. Previous studies suggested poor oral hygiene, concomitant diseases such as diabetes mellitus, low quality of life, and less frequent dentistry check-up as a probable explanation for this difference.[14] On the other hand, there was not any significant difference between the three groups in terms of bacteria causing periodontal infections (*Porphyromonas*). Although its isolation abundance was higher in the hemodialysis group compared to the two other groups, this difference was not statistically significant. Takeuchi and colleagues[6] and Castillo and coworkers[14] found that *Porphyromonas* is significantly higher in patients on hemodialysis than in controls.

CONCLUSIONS

Hemodialysis and kidney transplantation could affect the oral microflora. The isolation of *Candida* was more significant in patients on hemodialysis and in kidney transplant recipients in comparison to healthy individuals, which might be due to the immunodeficiency in these patients. *Streptococcus mutans*, *Lactobacilli*, *Porphyromonas*, and *Candida* are less seen in the oral microflora of kidney transplant recipients in comparison with patients on hemodialysis. Although this difference is not significant, it might be in favor of promising effect of kidney transplantation on oral microflora.

CONFLICT OF INTEREST

None declared.

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