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CLINICAL RESEARCH

Comparative evaluation of bacterial colonization on removable dental prostheses in patients with COVID-19: A clinical study

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Removable dentures are still popular, especially in economically disadvantaged communities because of their cost-effectiveness. However, denture wearing can gradually alter the oral ecology, especially oral microorganisms, and may provide the conditions for the invasion and colonization of pathogenic microorganisms.¹ The emergence of such conditions for oral and dental microorganisms and microflora can result in denture-related lesions such as denture stomatitis.²

With the advent of the COVID-19 pandemic, many concerns about the consequences of this viral disease have been identified, including its potential oral and dental complications.^{3,4} Oral manifestations of this infection include palate stomatitis and ulcers, halitosis with generalized erythematous and edematous gingivae, and necrotic interdental papillae with unprovoked gingival bleeding.^{5,6} Additionally, COVID-19 infection may have a detrimental effect on denture wearers, creating conditions for further colonization of opportunistic bacteria and changes in the oral microflora.⁷ However, studies that evaluated such detrimental effects are sparse. The present study assessed microbial

ABSTRACT

Statement of problem. In the outbreak of COVID-19, coinfections and even superinfections in the background of SARS-CoV-2 viral infection have been reported. Such bacterial and fungal strains may be colonized in different tissues and organs, including the oral cavity. Whether infection with COVID-19 could increase colonization of different bacterial strains on removable dental prostheses is unclear.

Purpose. The purpose of this clinical study was to compare bacterial colonization on removable dental prostheses in patients with COVID-19, before versus after diagnosis.

Material and methods. Two sex- and age-matched groups of complete-denture-wearing participants (N=60) with and without a positive diagnosis for COVID-19 were enrolled in the study. Swabs were used at 2 different time intervals to sample areas of the dentures, which were then cultured and the colony smears Gram stained. A statistical analysis was conducted by using the Mann-Whitney U test ($\alpha=.05$).

Results. *Streptococcus* species (93.3% versus 40.0%, $P=.047$) and *Klebsiella pneumonia* (46.7% versus 13.4%, $P=.036$) were detected more frequently in the COVID-19-positive group.

Conclusions. Higher rates of bacterial colonization, especially with *Streptococcus* species and *Klebsiella pneumonia*, were detected on removable dental prostheses after COVID-19 infection. (J Prosthet Dent 2021;■:■-■)

contamination of removable dental prostheses in adults diagnosed with COVID-19 and then compared this condition with a similar time period in the year before the occurrence of the COVID-19 pandemic. The null hypothesis was that denture wearers with COVID-19 are not prone to other microbial infections.

MATERIAL AND METHODS

In this pilot study, 2 sex- and age-matched groups of denture wearers at 2 different time intervals were enrolled in the study (N=60). The sample size provided a

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Clinical Implications

Denture wearers with COVID-19 infection appear to be more prone to other microbial infections and, therefore, may require additional evaluation and treatment.

statistical power of 80% and a confidence interval of 95%. The first group (n=30) was recruited from patients referred to the Yasoj Dental clinic between January and April 2019 for their first removable complete dentures. The routine protocol in this clinic has been swab sampling to evaluate bacterial microorganisms on the intaglio surface of the prosthesis 6 months after prosthesis placement. Therefore, participants with complete information about their bacterial evaluation were included in the study. Patients with systemic disorders or severe infections requiring antibiotic therapy were excluded from the study. The second group (n=30) was recruited from patients who received removable complete dentures between January and April 2020 and had been diagnosed with COVID-19 disease. These diagnoses were made after tracing for 3 months subsequent to the emergence of clinical manifestations associated with COVID-19 disease followed by molecular evaluation for the SARS-CoV-2 virus. Patients with evidence of severe disease, the need for hospitalization, evident lung involvement on computed tomography (CT) scan, or the need for antibiotics were excluded from the study. These participants had also received swab sampling of the denture surface within 3 months of the diagnosis of COVID-19. Individuals in either group receiving specific medications or reporting subjective symptoms of systemic or oral diseases were excluded.

To collect samples from the denture surface, a sterile cotton swab impregnated with phosphate buffer salt (PBS) was rubbed on the intaglio surface and then inserted in the tube containing PBS and transferred immediately to a microbiology laboratory. All assessments were carried out in a single laboratory in contract with the clinic. After shaking, the collected swab was inoculated onto the MacConkey agar and blood agar plates with a sterile pipette. The plates were then incubated for 48 hours at 37 °C and assessed for microbial growth, and the colony smears were prepared and Gram stained. The strains of microorganisms were identified by using specific biochemical tests and counted by a calibrated microbiologist (F.K.) using a colony counter (Sterlitech, Spatz).

The results were presented as mean \pm standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Categorical variables were

Table 1. Frequency of different bacterial strains on removable denture surface

Strain	Sampling in 2019 (Participants Without COVID-19), N=30	Sampling in 2020 (Participants With COVID-19), N=30	P
<i>Streptococcus</i> species	12 (40.0%)	28 (93.3%)	.047
<i>Staphylococcus aureus</i>	12 (40.0%)	19 (63.3%)	.306
<i>Candida albicans</i>	6 (20.0%)	8 (26.7%)	.630
Coagulase-negative <i>Staphylococcus</i>	4 (13.4%)	7 (23.3%)	.405
<i>Klebsiella pneumonia</i>	4 (13.4%)	14 (46.7%)	.036
<i>Diphtheroids</i>	2 (6.7%)	6 (20.0%)	.266
<i>Escherichia coli</i>	2 (6.7%)	6 (20.0%)	.0266
<i>Micrococcus</i> species	2 (6.7%)	3 (10.0%)	.667
<i>Lactobacillus</i> species	1 (3.3%)	4 (13.4%)	.358
<i>Pseudomonas aeruginosa</i>	0 (0.0%)	1 (3.3%)	.998
<i>Enterococcus</i>	0 (0.0%)	1 (3.3%)	.998

compared by using the chi-square or Fisher exact test when more than 20% of cells with an expected count of less than 5 were observed. Quantitative variables were also compared with the *t* test or the Mann U test. A statistical software program (SPSS v16.0 for Windows; SPSS Inc) was used for the statistical analyses ($\alpha=.05$).

RESULTS

The 2 groups of participants at the 2 different time periods (2019 and 2020) were matched for mean age (62.2 \pm 7.6 years versus 61.4 \pm 6.42 years, $P=.656$) and male sex (73.3% versus 70.0%, $P=.779$). The frequency of bacterial strains found in the samples of the 2 participant groups is presented in Table 1. A higher detection rate of *Streptococcus* species (93.3% versus 40.0%, $P=.047$) and *Klebsiella pneumonia* (46.7% versus 13.4%, $P=.036$) was found in the COVID-19 group. The risk of colonization for *Streptococcus* species on the removable denture surface after being diagnosed with COVID-19 increased by 2.33 times (95% CI: 1.01 to 5.43), and for *Klebsiella pneumonia* by 3.50 times (95% CI: 1.03 to 11.87).

DISCUSSION

The present study determined that patients with COVID-19 infection were significantly more prone to other microbial colonization, so the null hypothesis was rejected. Coinfections and even superinfections have been reported to be associated with SARS-CoV-2 viral infection.^{8,9} Several bacterial pathogens appear to be prevalent as causative agents of secondary infections, including antibiotic-resistant strains of *Staphylococcus aureus* and

Klebsiella pneumoniae.¹⁰ Most of these bacterial infections occurred in hospitalized patients with COVID-19, exacerbating the poor clinical outcome. However, the occurrence of bacterial or even fungal infection in individuals with less severe COVID-19 infection had also been expected because of the reduced immune response in these patients, especially in the elderly.¹¹ Such bacterial and fungal strains may be colonized in different tissues and organs even in the oral cavity.¹² Infection with COVID-19 could increase colonization of these strains on dentures with unwanted consequences. Although studies have been published on coinfection of SARS-CoV-2 and opportunist bacteria, especially in hospitalized patients with COVID-19, few have focused on coinfection with bacterial colonization of oral and dental tissues. As specified by Dos Santos et al,¹³ oral and dental lesions in patients with COVID-19 may not be a primary result of invasion by SARS-CoV-2 into ACE-2 receptors on the oral mucosa but may be secondary to poor baseline immunity and superinfection due to other microorganisms. Chakraborty¹⁴ also presented evidence on the higher colonization of cariogenic and periodontopathic bacteria in patients with COVID-19, endorsing the notion of a connection between the oral microbiome and COVID-19 complications. Evidence suggests that periodontal pathogenic bacteria are involved in the pathogenesis of respiratory diseases, such as those implicated in COVID-19.¹⁴

The present study had limitations such as the small sample size and short follow-up time. Future studies should evaluate the same study concept with a higher sample size and longer follow-up time with more strict inclusion criteria.

CONCLUSIONS

Based on the findings of this clinical study, the following conclusions were drawn:

1. Patients with COVID-19 may experience increased bacterial colonization on dentures, even in its early and mild stages.
2. This increase in opportunistic bacterial rates could exacerbate the clinical manifestations of COVID-19 disease such as respiratory distress syndrome and even fatal pneumonia and therefore could be a warning sign for patients with early COVID-19 disease.

3. Increasing the colonization of these bacteria may accelerate the degradation of the dentures and may be associated with damage to dental or oral tissue.
4. The frequent evaluation and sampling of dental prostheses in patients diagnosed with COVID-19 is recommended from the beginning stages, as well as the use of appropriate medications to prevent the invasion of opportunistic bacteria.

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