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RESEARCH ARTICLE

THE EFFECT OF DENTAL IMPLANTS ON INCREASING THE COLONIZATION RATE OF AEROBIC BACTERIA IN THE ORAL CAVITY

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Abstract

Background and aims: Dental implants' principal function is to support artificial teeth. The physiological process by which bones firmly adhere to the surface of various ceramics and metals, such as titanium, is what leads to the emergence of modern dental implants, and there may be negative effects of these implants on the balance in the bacterial numbers in the mouth. Therefore, this study compared the colony forming unit (CFU) of oral bacteria from the buccal mucosa and buccal tongue between patients who had dental implants and healthy volunteers without dental implants.
Methods: In this study, 36 people with dental implants and 36 people without dental implants were both included. Following serial dilutions were made and distributed on blood agar, samples were grown in Brain Heart Infusion Broth (BHI). When a single layer of bacteria developed on blood agar at any dilution level, CFU was estimated. Version 7 of Epi-info Statistics software was used to analyze the data.
Results: For non-implant controls the values for buccal mucosa of bacterial counts were slightly lower than that of the implant patient's buccal mucosa. There was a significant correlation between the increase of aerobic bacterial colonization of the tongue with the implants where the mean±SD was 196.8±12.9 CFU/ml greater than 183.4±9.1 CFU/ml for the normal controls; indicating the enhancement of the effect of the implant in the heavy colonization of bacteria in the oral cavity among implant patient group (p<0.0001). Additionally, there was a strong correlation between the duration of 13–24 months since implant placement and decreased bacterial colonization of the oral cavity with the mean±SD being 193.2±10.3 CFU/mL vs. 209.6±13.8 CFU/mL for ≤ 12 months; p=0.005.
Conclusion: Patients with implants had greater lingual buccal tongue CFU readings than non-implant patients, suggesting that implants are more prone to plaque adhesion. Dental implants, particularly those associated with five implants or more and those recently placed, increased the amount of bacteria leading to heavily colonized of the oral cavity.
Keywords: Dental implants, lingual buccal tongue CFU, normal teeth, oral bacterial colonization, Yemen.

INTRODUCTION

Dental implants are mostly used to support artificial teeth. Modern dental implants were made possible by a physiological process that permits bone to firmly bind to the surface of particular materials, such titanium and some ceramics. The union of the implant and bone can sustain physical loads for decades without fracturing[1]. In Yemen and throughout the world, dental implants are extensively used. As an illustration, dental implants were used by 0.7% of patients in the United States who had lost at least one tooth in 1999–2000, 5.7% in 2015–2016, and are expected to be used by more than 26% of patients in 2026. Single missing teeth (single

tooth restorations), multiple missing teeth (multiple tooth restorations), or single or multiple missing teeth (implant-retained fixed bridge, implant-supported over-denture) are all treated with dental implants[3]. In orthodontics, dental implants also referred to as orthodontic micro implants are utilized to provide anchoring when orthodontic treatment is necessary[4] before a dental implant is inserted. Obturators, a removable prosthesis designed to block the hole between the oral cavity and the maxilla or nose, are being maintained with implants more and more frequently[3]. Facial prostheses can link to implants inserted in the facial bones to address facial abnormalities (caused, for example, by trauma or cancer treatment).

The implant can be used to hold a fixed or removable prosthesis to replace a portion of the face, depending on the circumstances[5]. All of the aforementioned points point to the value, wide use, and capability of the dental implant in the clinical field. In addition, the implant is regarded as one of the most popular dental procedures available today[5]. Implants deteriorate due to the loss of supporting bone and soft tissue, which is where, signs of mobility appeared[6]. Implant failure occurs when infection in the gingival margin appears similar to gingivitis implants, and bacterial infection may act as pockets around the implants[7]. Therefore, information or research on conditions related to the soft tissues around the implant should not be neglected. Implant periarthritis was defined as an inflammatory condition involving the dental implant, surrounding mucosa, and bone that may lead to loss of supporting bone[8].

The basis of dental implant collapse has been linked to anatomical factors, surgical procedures, and bacterial contamination of the implant[9]. It is also known that implant failure is due to inflammation of the implant periphery, because the presence of pathologies on the implant surfaces has an important role in potentially influence the osseointegration of the implant into the adjacent bone[10]. Successful implant practice depends on the nonexistence of inflammation in the oral cavity and peri-implant tissue, two areas where micro-organisms may inhabit the implant surface[11]. Normal oral bacteria, which inhabit the oral cavity, are known to cause dental diseases such as caries and periodontal disease. Each human may possess more than 150 distinct microbial species, with the ability to colonize dentate estimated to be 400 different microbial species in the oral cavity[6]. Compared to any other area of the body, the mouth offers bacteria a conducive environment for growth and multiplication[12].

This study compared the colony forming unit (CFU) of oral bacteria from the buccal mucosa and buccal tongue in patients who had dental implants and in healthy volunteers without dental implants.

MATERIALS AND METHODS

Bacterial tests were performed on 72 individuals (36 dental implant patients and 36 individuals with natural teeth) over the course of three months, starting in December 2022 and ending in February 2023, in the dental clinics of the Faculty of Dentistry, Sana'a University, Yemen, and private dental clinics (Al-Mortadda Dental Clinics, Al-Kahara Dental Clinics).

Sample Size and power

The sample size was determined using calculation software based on comparing between rate of variation between cases (dental implant patients) and controls. If the ratio of change of CFU counts for control group is 2% and for cases is 12%, with 99.9% confidence level, power equal to 0.05, we need 36 subjects in each group. A total of 72 subjects were selected (36 subjects with a dental implant and 36 subjects without the dental implant).

Collection of Patient Sample for aerobic bacterial count (CFU): Each patient and control had two sterile cotton swabs drawn from them to collect samples. Swab samples from both groups were placed in Stuart transport medium before being delivered to the microbiology lab. At two separate points in the mouth of the patient, buccal mucosa and lingual buccal tongue mucosa oral swabs were taken[13-15].

Bacterial Culture

BHI Broth: The most popular culture medium for this type of bacteria is called Brain Heart Infusion Broth (BHI Broth).

Blood-Mueller-Hilton Agar Preparation

Niacinamide adenine dinucleotide and five percent sheep blood should be added. Given that it is a non-selective, non-differential media, nearly every organism plated on it grew, and starch in the medium absorbs toxins produced by bacteria. In 1 L of filtered water, 38 grams of the powder were suspended. Combine thoroughly, heat with constant stirring, and boil for one minute to completely dissolve the powder. The medium was autoclaved at 121°C for 15 minutes, cooled to 45–50°C, and then 5% sterile defibrinated sheep blood was aseptically added. In order to achieve a uniform depth of 4 mm (60–70 mL from the center for 150 mm plates and 25–30 mL for 100 mm plates), the cooled agar was then placed onto sterilized Petri dishes on a flat, horizontal surface.

Inoculation of Bacterial: The BHI Broth sample that was taken was incubated for 24 hours at a constant temperature of 37°C.

Bacterial Dilution: Although the maximum density varies significantly based on the species of bacteria and the substrate in which they are cultivated, bacteria normally grow at varying densities. Therefore, a variety of dilutions should be created and each of them should be titrated with one or two dilutions to generate easily countable numbers of bacteria. There were created ten-fold serial dilutions of all-covering bacteria. 0.1 mL of each dilution was then transferred and placed over a blood agar plate that had been prepared. Blood agar was used to grow samples in duplicate. The culture medium was then incubated at 37°C for 24 to 48 hours.

Colony Forming Unit (CFU): Plates were counted (or repeat plates from the same dilution) with only 30-300 colonies and more than 300 colonies plate was reject.

Statistical Analysis: Version 7 of Epi-info Statistics software was used to analyze the data. In each graph, as well as all results were expressed as mean standard

error of the mean (SEM) in the table's average and standard deviation (SD). The data's normal distribution was determined (p>0.05) and confirmed as such using the Shapiro-Wilk normality test. For determining homogeneity or uniformity of variance, Levene test results were observed (homogeneous for p>0.05). To compare the means of CFU oral bacterial between the control and case groups from buccal mucosa and lingual mucosa, an independent- t test was used. The data gathered was normally distributed. A colony-forming unit (CFU) is a unit used to describe how many colonogenic cells are viable in a milliliter of solution. These provide a rough estimate of the number of cells that are still viable, capable of dividing, and forming small colonies. CFU/ml is determined by multiplying the total number of colonies by the dilution factor and dividing the result by the size of the culture plate[13].

Ethical Consideration: The Contract No. 177 project received ethical approval from the Faculty of Medicine and Health Sciences at Sana'a University's Medical Ethics and Research Committee, on October 20, 2022. The review committee's ethical guidelines were adhered to at all times. The selected subjects gave their signed, informed consent.

RESULTS

The study included 36 dental implant individuals, 61.1% male and 38.9% female, ranging in age from 37-62 years, with a mean±SD of age equal to 49.5±6.8 years old. Most of the participants were in the age group 46-55 years (52.8%) (Table 1). Regarding number of implant, the mean±SD of implants is equal to 3.3±0.91 implants. Most of the participants had 3 implants (41.7%) and 30.6% had 4 implants. Regarding duration of the implants most of the participants have implant between 13-24 months (61.1%) with a mean± SD of duration equal to 17.8±6.5 months. Table 2 shows the CFU/ml of the tongue and buccal mucosa of the oral rate of bacterial colonization of the case group (with dental implant) compared to healthy controls. For implant patients, in all, the mean±SD of the tongue bacterial count was 196.8±12.9 CFU/mL, with mode equal to 188 CFU/mL, the median was 198.5 CFU/mL, and ranged from 171 to 230 CFU/mL with the interquartile range being 75% (IQR) equal to 203 CFU/mL; variance in all individual values was significantly distributed over the normal curve with a 91 t-test, and p<0.001.

Table 1: General characteristics of implant patients participate in the study.

Characters	Number
Sex	
Male	22 (61.1)
Female	14 (38.9)
Ages	
≤45	10 (27.8)
46-55	19 (52.8)
≥56	7 (19.4)
Mean age	49.5Years
SD	6.8 Years
Mode	51 Years
Median	51 Years
Min-Max	37-62 Years
Number of implants	
2 implants	7 (19.4)
3 implants	15 (41.7)
4 implants	11 (30.6)
≥5 implants	3 (8.4)
Mean±SD	3.3±0.91implants
Duration of implants	
≤ 12 months	8 (22.2)
13-24 months	22 (61.1)
≥25 months	6 (16.7)
Mean±SD	17.8±6.5 months

For implant patients, in all, the mean±SD of the buccal bacterial count was 198.2±13.4 CFU/mL, with mode equal to 189 CFU/mL, the median was 199.5 CFU/mL, and ranged from 175 to 235 CFU/mL with the interquartile range being 75% (IQR) equal to 205 CFU/mL; Variance in all individual values was significantly distributed over the normal curve with a 88.9 t-test, and p<0.001.

For non-implant controls the values were slightly lower than that of the implant patients; in all, the mean±SD of the tongue bacterial count was 183.4±9.79 CFU/mL, with mode equal to 178 CFU/mL, the median was 182.5 CFU/mL, and ranged from 166 to 205 CFU/mL with the interquartile range being 75% (IQR) equal to 189.5 CFU/mL; variance in all individual values was significantly distributed over the normal curve with a 120 t-test, and p<0.001.

Table 2: CFU/ml oral bacterial buccal and tongue mucosa for the case group (with implant) comparing with healthy controls.

Characters	Implant patients CFU/ml		Normal controls CFU/ml	
	Tongue counts	Buccal counts	Tongue counts	Buccal counts
Mean	196.8	198.2	183.4	185.1
SD	12.9	13.4	9.1	9.79
SE	2.1	2.22	1.5	1.63
Min	171	175	166	165
Max	230	235	205	212
Mode	188	189	178	187
Median	198.5	199.5	182.5	184.5
25% ile	188	189	177.5	178
75% ile	203	205	189.5	189.5
T-test	91	88.9	120	113
Df	35	35	35	35
p-value	<0.0001	<0.0001	<0.0001	<0.0001

Table 3: Mean±SD of CFU/ml oral bacterial buccal and tongue mucosa for the case group (with implant) comparing with healthy controls and the significance of variations.

Sites	Implant patients	Normal controls	Difference	*SE*	*95% CI*	*t*-test	*DF*	*p*
	Mean±SD	Mean±SD						
Tongue counts	196.8±12.9	183.4±9.1	13.4	2.6	-18.6 to -8.1	-5.1	70	<0.0001
Buccal counts	198.2±13.4	185.1±9.79	-13.1	2.77	-18.6 to -7.5	-4.7	70	<0.0001

For non-implant controls the values for buccal mucosa of bacterial counts were slightly lower than that of the implant patient's buccal mucosa (Table 2). Table 3 shows the mean±SD of CFU/ml bacterial mouth buccal and tongue mucosa of the case group (dental implant) compared with healthy controls and the significance of the differences. There was a significant correlation between the increase of aerobic bacterial colonization of the tongue with the implants where the mean±SD was 196.8±12.9 CFU/ml greater than 183.4±9.1 CFU/ml for the normal controls; indicating the enhancement of the effect of the implant in the heavy colonization of bacteria in the oral cavity among implant patient group (p<0.0001). When considering the association between sexes, age, number of implants, and duration of implants; and promoting bacterial colonization of the tongue and buccal mucosa. There was a significant association between ≥5 implants and heavy bacterial colonization of the oral cavity with the mean±SD being 214±13.9 CFU/mL versus 192.85±4.44 CFU/mL for the two-implant composite patient; p=0.027. Also, there was a significant association between the duration of 13–24 months since implant placement and decreased bacterial colonization of the oral cavity with the mean±SD being 193.2±10.3 CFU/mL vs. 209.6±13.8 CFU/mL for ≤ 12 months; p=0.005. However, there was no significant association between heavy or light colonization and the sex and age of implants.

Table 4: The mean±SD with general characteristics of implant patients participate in the study (tongue and buccal).

Characters	Tongue		Buccal	
	Mean±SD CFU/ml	*p* value	Mean±SD CFU/ml	*p* value
Sex				
Male n=22	196±11.9	0.64	197.6±13.1	0.74
Female n=14	198.1±14.7		199.1±14.2	
Age group (years)				
≤45 n=10	193.1±9.8	Control	194.5±10.8	Control
46-55 n=19	198.8±15.7	0.307	200.2±15.9	0.3
≥56 n=7	196.7±6.99	0.41	198±8.4	0.47
Number of implants				
2 implant n=7	192.85±4.44	Control	193.3±11.3	Control
3 implants n=15	192.3±12.8	0.92	195.03±13.97	0.78
4 implants n=11	200.8±9.01	0.04	201.7±8.23	0.08
≥5 implants n=3	214±13.9	0.0047	216.7±15.9	0.027
Duration of implants				
≤ 12 months n=8	209.6±13.8	Control	210.1±16.1	Control
13-24 months n =22	193.2±10.3	0.0016	194.95±10.5	0.005
≥25 months n=6	192.8±10.6	0.03	194.2±11.2	0.06

This procedure calculates the difference between the observed means in two independent samples. A significance value (p-value) and 95% Confidence Interval (CI) of the difference is reported. The p-value is the probability of obtaining the observed difference between the samples if the null hypothesis were true. The null hypothesis is the hypothesis that the difference is 0[26].

DISCUSSION

Bacterial colony quantity was utilized in this study as a marker to represent the condition of the oral cavity with measurement of CFU as performed by previous studies[13-16]. A colony-forming unit (CFU) in micro-biology is a measurement that determines how many viable, competent, and reproducible microorganisms are present in a sample under specific, controlled conditions. The goal of plate counting is to determine the quantity of cells by counting the number of colonies that can form when particular nutrient medium, temperature, and time conditions are met. A colony might theoretically develop from a single viable cell through the process of replication. The progenitor of the colony, however, was probably a collection of cells that were deposited together because solitary cells are the uncommon in nature. Therefore, our results were approximately representative of the actual number of aerobic bacteria, and the CFU method was suitable for testing the theory of the study. Despite coming from various racial/ethnic groupings, the other study published a CFU reading of normal patients without any implants inside the oral cavity is fairly comparable to this study of subjects without implants[17]. Although there is a chance of inaccuracy when counting colony growth on an agar plate, especially if the growths appear to be multi-layered, this study used bacterial dilutions up to five times to avoid this issue as stated previously[13,18].

In the current study, there was a significant correlation between the increase of aerobic bacterial colonization

of the tongue with the implants where the mean±SD was 196.8±12.9 CFU/ml greater than 183.4±9.1 CFU/ml for the normal controls; indicating the enhancement of the effect of the implant in the heavy colonization of bacteria in the oral cavity among implant patient group (p<0.0001). This result is consistent with the fact that depending on the level of oral hygiene, the pattern of bacteria that colonized the mouth cavity varied among various hosts and the presence status of dental prostheses such as dentures, orthodontics, etc[13,14]. Gram-negative and anaerobes have been discovered in individuals with poor oral hygiene or in those who have dental prostheses like dentures or orthodontics[15,19], although a normal, healthy oral cavity primarily exhibits Gram-positive aerobic proliferation. Also, the oral cavity is the diverse microbial community present on the tooth surface and salivary origin[20]. It was found that the infection around the implant comes from the patient's mouth, and the infection may have been due to the increase in the numbers of bacteria on the implanted tooth plate[12]. Therefore, by giving the dentist an evidence-based result that is helpful in treatment maintenance after installation, understanding the pattern of microorganisms colonizing in the oral cavity of a dental implant patient is vital in maintaining the longevity of implant therapy. When considering the association between number of implants; and promoting bacterial colonization of the tongue and buccal mucosa. There was a significant association between ≥5 implants and heavy bacterial colonization of the oral cavity with the mean±SD being 214±13.9 CFU/mL versus 192.85±4.44 CFU/mL for the two-implant composite patient; p=0.027. This result can be explained by the fact that the initial adhesion and colonization of bacteria on the implant surface is considered to be high and could play a major role in the pathogenesis of infections related to biomaterials[21]. In normal teeth or an intact oral cavity, bacteria are prevented from attaching to the periodontal tissue as they are physically limited by the gingival mucosa, which forms a seal around the neck of the tooth[22] whereas for dental implants the gingival mucosal formation is less possible to form. Intact, attached gingiva and crevicular fluid, also known as biological width, which may be negatively impacted by implants, are the additional natural preventive measures that stop the entry of the microbe into periodontal tissue.

Implant longevity is influenced by a number of variables, including oral cavity health, which is closely tied to the amount of bacteria that has colonized and may be harmful to the oral tissue and implant. In order to enhance and optimize the maintenance care and guarantee the survival and longevity of the dental implant, it is necessary to know how much bacteria are present in the oral cavities of patients who have dental implants.

Also, current result could be explained by that, an increase in the CFU read highlighted more bacteria colonies, while a decrease in the CFU read would represent a lower bacterial colony as described by other authors[13,24]. More bacteria colonies or a rise in CFU readings would indicate poorer oral hygiene than the common perception; however, prior research[13,14,19,25] has shown that understanding the exact type of bacteria colonies is more significant than the presence of colonies. The presence of the prosthesis as a superstructure for the implant within the oral cavity has an impact on the difference in oral hygiene status or condition. While the smoother surface of the prosthesis is better for cleaning, the rougher surface will hold more plaque. If plaque buildup is not prevented by appropriate dental hygiene practices, bacteria can proliferate because the plaque is imbedded in bacterial colonies[13].

There is only one recent study that discussed the effect of implants on oral bacterial colonization[13], but the results for this study were different from the current study as the previous study had shown that there was no significant effect of implants on oral bacterial colonization as the numbers were almost the same in both groups, implant group and normal healthy control group. In the current study, the amount of bacterial colonies was significantly different between the implant and non-implant CFU readings. This means that the effect of the superstructures present in the implant fixtures on the bacteria colonies is different from the presence of the teeth in the normal control group. These findings are not supported by other articles that stated that implant-supported crowns are the most widely accepted treatment in modern dentistry, and are similar to natural teeth in many aspects, such as functionality, comfort, and maintenance[13,26].

Limitation of the study: There is need of *in-vivo* study for the estimation of the effectiveness of the prepared formulations.

CONCLUSIONS

The significant difference in bacterial load between implant patients and non-implant patients suggests that the presence of implants in the oral cavity may interfere with or deteriorate oral health. However, this effect is different from that of natural teeth because implants do not directly affect the surrounding oral flora like actual teeth do. Additionally, patients with implants had greater lingual buccal tongue CFU readings than non-implant patients, suggesting that implants are more prone to plaque adhesion. Dental implants, particularly those associated with five implants or more and those recently placed, increased the amount of bacteria leading to heavily colonized of the oral cavity.

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AUTHOR'S CONTRIBUTIONS

Sharafuddin AH: writing, analyzed data. **Alshameri BH:** data analysis, report drafting. **AL-Haddad KA:** editing, review. **Al-Najhi MMA:** data analysis and

interpretations. **Al-Shamahy HA:** editing, supervision. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

Regarding this project, there is no conflict of interest.

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