DocID: https://connectjournals.com/03896.2020.20.3755

*Biochem. Cell. Arch.* Vol. 20, Supplement 2, pp. 3755-3760, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

# COMPARISON OF PERI-IMPLANT MICROBIOTA BETWEEN HEALTHY AND DISEASED IMPLANTS

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**(Received 6 January 2020, Revised 29 March 2020, Accepted 17 May 2020)**

**ABSTRACT : Recently, dental implants have experienced increasing demand as one of the most effective, permanent and stable ways for replacing missing teeth. However, peri-implant diseases that are multispecies plaque-based infections may ultimately lead to implant failure (*i.e.*, late peri-implantitis). Therefore, the present study aims to detect the microbial diversity of subgingival plaque in peri-implantitis cases (N = 30) by comparing with healthy implants (N = 34) using culture-based identification methods, including VITEK 2 system. An increase in microbial diversity (29 species along with 1 and 7 isolates, which were classified as a genus and unidentified species, respectively) were observed in subgingival sites of diseased implants dominated by Gram negative enteric bacilli compared with healthy implants (21 species with 2 at genus level) with the majority of Gram-positive lactic acids species. Our results showed significant differences in the mean age between healthy (53.14±11.34) and diseased implants (61.9±9.71).**

***Key words :*** Peri-implantitis, microbiota, dental implant.

## INTRODUCTION

The dental implant is an artificial device usually made of titanium, inserted into the bone for replacing one or more missing teeth (Branemark, 1985). Peri-implantitis is a destructive pathological inflammatory process affecting the soft and hard tissues surrounding dental implants. The soft tissues become inflamed whereas the alveolar bone, which surrounds the implant for the purposes of retention, is lost over time. Peri-implantitis is similar to periodontitis as infectious diseases (Lindhe and Meyle, 2008).

Microbial infections with bacteria,possibly viruses and yeasts play an important role in the disease progression (Verdugo *et al*, 2015). Quirynen and Van Assche (2011) detected high levels of bacteria related with periodontitis and peri-implantitis, in totally edentulous patients, agreeing with other similar studies of Quirynen *et al* (2005), Devides and de Mattias Franco (2006) and Sachdeo *et al* (2008).

Slots *et al* (1991) also reported that in implants with peri-implantitis, it is possible to detect big quantities of Gram-negative anaerobic bacteria, including Fusobacteria, spirochetes, *B. forsythus*, *P. intermedia*, *P. nigrescens* and *P. gingivalis*. *Aggregatibacter actinomycetemcomitans* was also isolated in this type of lesion.

*A. actinomycetemcomitans* and *P. gingivalis* were found in large quantities in peri-implant lesions. These two pathogens can be considered the predominant microorganisms, being responsible for destructive infectionin peri-implantitis (Heydenrijk *et al*, 2002; Botero *et al*, 2005). Also, Van de Velde *et al* (2009) have demonstrated the presence of *A. actinomycetemcomitans*, *Fusobacterium* sp., *P. gingivalis*, *P. aeruginosa* and *T. forsythia*, in implants diagnosed with peri-implantitis. Symbiosis between *Bacteroides* sp. and *P. aeruginosa* seems to favor the persistence of *P. aeruginosa* in inflamed regions around implants.

For several years, there may be a balance between the challenged bacteria and host peri-implant tissues, the formed biofilm is in a symbiotic state and this homeostatic state indicates the state of health around the implant (Marsh, 1994 and 2003).

In peri-implantitis cases, biofilm has high amounts of Gram-negative bacteria, as well as Gram positive cocci (*Parvimonas* sp. and *Peptostreptococcus* sp.) (Koyanagi *et al*, 2013).

Peri-implantitis is considered an infectious disease requiring antimicrobial therapy to target specific putative bacteria. Peri-implantitis usually requires surgical treatment. Many clinicians have recommended subgingival irrigation of the peri-implant space with antiseptic agent (Roos-Jansaker *et al*, 2007).

**Aim of the study :** Comparison the common and important bacterial species associated with diseased and healthy implants.

## MATERIALS AND METHODS

A total of 71 participants, thirty-four cases with healthy implants and 37 patients with diseased implants (They were diagnosed with late peri-implantitis) were collected from different private dental clinics, Baghdad, Iraq in 10 months. Full medical history was recorded in a case sheet for each patient, which is designed to include some possible risk indicators for peri-implantitis. All clinical examinations were performed by periodontist.

### Inclusion and exclusion criteria

Specimens were collected from patients, if they had at least one dental implant with definitive prostheses for at least one year and the implant of the patient was diagnosed with peri-implantitis, evident in radiographic bone loss ≥3 mm, PPD ≥6 mm and a positive BoP score (Renvert *et al*, 2018). While some patients were excluded if they have had any follow-up visit for plaque control of the prosthesis and/or the implants, patients who had not taken any antibiotic or anti-inflammatory therapy in the past 6 months prior to clinical examination and sampling, patients under chemo, radiation therapy, patients with allergy to Metronidazole (MTZ) and/or amoxicillin (AMX), patients got orthodontic intervention and patients who had poorly controlled diabetes mellitus (HbA1c ≥8.0) (Schuldt Filho *et al*, 2014).

### Samples collection

According to Bathla (2012), subgingival plaque samples were collected for microbiological analysis as following :

1. Before sampling, patients were instructed to refrain from food for 2 hours and oral hygiene (brushing or flossing the teeth) for 12 h before sampling.
2. Pre-secreted saliva was removed by rinsing the oral cavity with sterile normal saline for 3 times.
3. Visible supra-gingival plaque and calculus deposits were carefully removed by a sterile curette in a coronal direction to avoid pushing supra-gingival plaque into subgingival space.
4. The residual plaque was removed by wiping with sterile gauze pads soaked in saline.
5. The experimental areas (*i.e.* implant abutments or prostheses) were isolated with sterile cotton rolls to avoid saliva contamination and gently dried the external surface of the soft tissues with an air syringe.
6. Subgingival plaque was sampled by placing a sterile nickel-plated curette to the depth of the peri-implant sulcus/peri-implant pockets and moved coronally with firm lateral pressure against the root surfaces. The material is then immediately dislodged from curette tip into the 10 ml screw cap tube containing thioglycolate broth as transport medium and incubated with anaerobic candle jar.

### Isolation and identification

Pattern of aerobic, anaerobic and facultative anaerobic bacteria were done employing standard bacteriological techniques (Prescott *et al*, 2014):

1. The tubes containing samples were pre-incubated for 30 minutes at 37°C and shaken vigorously in a vortex mixer for the 60s.
2. Ten fold serial dilutions of the samples were prepared in peptone water (Cogulu *et al*, 2007).
3. Bacteria were isolated by spreading diluted samples (100ìl) on Petri dishes containing brain heart infusion agar (BHIA) supplemented with 5% sheep blood agar.
4. All plates were incubated 37°C for 1-5 days aerobically and anaerobically by using anaerobic jar with the Gas-Pak for the strict anaerobic bacteria and anaerobic jar with the using of candle for the facultative and microaerophils bacteria.
5. Bacterial isolates were purified by sub-culturing on the same media used for isolation.
6. Each colony with different morphology on BHIA was isolated, sub cultured and identified (Prescott *et al*. 2014).
7. Preliminary identification of the bacterial isolates was conducted according to the morphological and cultural characteristics. For further identification, biochemical tests were used according to Bergey's Manual of Systematic Bacteriology (Vos *et al*, 2011).
8. To confirm the identification, all suspected bacterial isolates were subjected to VITEK 2 system.

## RESULTS AND DISCUSSION

Subgingival plaque was analyzed from 30 diseased patients and 34 healthy people using culture-based methods and VITEK 2 system for further identification.

The inclusion and exclusion criteria were applied to 37 patients suffering from peri-implantitis, thirty patients

met the selected inclusion criteria (mentioned previously in the material and methods section) and diagnosis with peri-implantitis in at least one dental implant and comprised the test group, 19 males (63.3%) and 11 females (36.7%) with a mean age of 62 ± 9.37 years (age range 43–77), while 34 participants represented the control group, with dental implants classified as being healthy, 15 (44.1%) males and 19 (55.9%) females with a mean age of 53.2 ± 11.34 years (age range 27-73) (Table 1).

**Table 1 :** Prevalence of diseased/ healthy implants among Iraqi patients.

| Implant status / Gender | Healthy implant | | | Diseased implant | | | Total (%) |
|---|---|---|---|---|---|---|---|
| | No. (%) | Mean age± SD | Age range | No. (%) | Mean age± SD | Age range | |
| Male | 15 (44.1) | 54.9±12.62 | 27-73 | 19 (63.3) | 62.21±9.27 | 43-77 | 34 (100%) |
| Female | 19 (55.9) | 51.7±10.34 | 34-72 | 11 (36.7) | 61.36±11.29 | 41-73 | 30 (100%) |
| Total (%) | 34 (100%) | 53.14+11.34 | 27-73 | 30(100%) | 61.9± | 9.71 | 41-7764 (100%) |

The *Z*-Score is -3.04721. The *p*-value is .00228. The result is significant at $p < .05$.

The result showed that there was a significant difference in age between the test group (61.9±9.71) and control group (53.14±11.34) p = 0.00228<0.05.

The degree of periodontal destruction increases with age (aging effect). With age, the slower the rate of wound healing, the higher the susceptibility to gum disease and the more speedily inflammation of the periodontium tends to develop (Van der Velden, 1984).

Increasing age can be considered as a risk indicator for developing peri-implantitis as many chronic systemic diseases are more common in elderly patients, which in turn can directly/indirectly affect the preimplant health (Mumcu and Fadhil, 2018). Consequently, as a leading factor for developing peri-implantitis when combined with other risk indicators such as periodontitis and history of cardiovascular disease (Serino and Ström, 2009; Park *et al*, 2017).

In a review of the 212 partial toothless group, the incidence of peri-implantitis in elderly individuals (> 45 years) was slightly higher (Ferreira *et al*, 2006). Elemek *et al* (2017) suggested that the probability of peri-implantitis was 3.2 times higher in individuals ≥ 60 years. This indicates that dentist's knowledge should be increased to prevent peri-implantitis. Nevertheless, it is also essential for patients to make regular dental checks within the supportive periodontal treatment period and to ensure the highest levels of oral hygiene (Mumcu and Fadhil, 2018).

Sixty-four subgingival plaque samples were recruited in this study, including 30 (46.9%) patients with peri-implantitis (test group) and 34 (53.3%) healthy individual (control group) for microbial analysis.

A total of 213 microbial isolates were obtained from the test group, about 194 (91%) of theses isolates fell into 30 species. Whereas, 12(5.6%) isolates were identified at one genus level (i.e. *Veillonella* spp.) and 7(3.3%) were considered as unidentified organisms.

On the other hand, the control group, thirty samples showed positive growth culture with a total of 148 isolates, only 18 (12%) of these isolates were identified at the genus level represented by7(4.7%), 6(4%) and 5(3.4%) for *Veillonella* spp., *Bifidobacterium* spp. and *Bacillus* spp., respectively. While, the rest (88%) were classified to the species level. The diversity of known species with respect to prei-implant health status is shown in Table 2.

As indicated from Table 2, seventeen bacterial species appeared exclusively in the test group (N=30) as follows : 18(60%) *Enterobacter cloacae* ssp. dissolvens, 11(36.7%) *Lactobacillus acidophilus*, 10(33.3%) *Acinetobacter baumannii*, 10(33.3%) *Pseudomonas aeruginosa*, 8(26.7%) *Enterococcus faecalis*, 6(20%) *Enterobacter aerogenes*, 6(20) *Campylobacter ureolyticus*, 5(16.7%) *Leuconostoc mesenteroides*, 5(16.7%) *Proteus mirabilis*, 5(7.8%) *Fusobacterium necrophorum*, 5(16.7%) *Serratia marcescens*, 4(13.3%) *Streptococcus pyogenes*, 3(10%) *Citrobacter freundii*, 2(6.7%) *Raoultella ornithinolytica*, 2(6.7%) *Sphingomonas paucimobilis*, 2(6.7%) *Klebsiella oxytoca*, 1(3.3%) *Pseudomonas fluorescens*, it should be noted that 7(23.3) isolates were considered as unidentified organisms by VITEK 2 system.

On the other hand, seven species and 2 *genera* only appeared in the control group (N=34), 10(29.4%) *Staphylococcus epidermidis*, 10(29.4%) *Lactococcuslactis* ssp. *cremoris*, 6(17.6%) *Bifidobacterium* spp., 6((17.6%) *Lactobacillus gasseri*, 5(14.7%) *Staphylococcus saprophyticus*, 5(14.7%) *Bacillus* spp., 3(8.8%) *Lactobacillus plantarum*, 2(5.9%) *Actinomyces naeslundii*, 1(2.9%) *Streptococcus pseudoporcinus*.

Taking into consideration that 4 samples from the control group showed no growth on culture media. It is

**Table 2 :** Isolation percentage from subgingival plaque of healthy and diseased implants with respect to isolated bacteria.

| Isolate \ Peri-implant status | | Healthy implant N=34, Tn = 148 No. | | | Diseased implant N = 30, Tn = 213 No. | | | Total (%) |
|---|---|---|---|---|---|---|---|---|
| | | Female | Male | Total (%) | Female | Male | Total (%) | |
| 1- | *Enterobacter cloacae* ssp. dissolvens | ND* | ND* | - | 8 | 10 | 18(60) | 18(28.1) |
| 2- | *Streptococcus thoraltensis* | 5 | 3 | 8(23.6) | 5 | 9 | 14(46.7) | 23(36) |
| 3- | *Veillonella* spp. | 3 | 4 | 7(20.6) | 1 | 10 | 12(40) | 17(26.6) |
| 4- | *Lactobacillus acidophilus* | ND* | ND* | - | 4 | 7 | 11(36.7) | 11(17.2) |
| 5- | *Escherichia coli* | 6 | 6 | 12(35.3) | 5 | 6 | 11(36.7) | 23(36) |
| 6- | *Acinetobacter baumannii* | ND* | ND* | - | 7 | 3 | 10(33.3) | 10(15.7) |
| 7- | *Pseudomonas aeruginosa* | ND* | ND* | - | 3 | 7 | 10(33.3) | 10(15.7) |
| 8- | *Candida albicans* | 2 | 3 | 5(14.7) | 4 | 5 | 9(30) | 14(21.9) |
| 9- | *Streptococcus mutans* | 4 | 3 | 7(20.6) | 3 | 5 | 8(26.7) | 15(23.4) |
| 10- | *Enterococcus faecalis* | ND* | ND* | - | 3 | 5 | 8(26.7) | 8(12.5) |
| 11- | *Staphylococcus hominis* | 6 | 4 | 10(29.4) | 1 | 7 | 8(26.7) | 18(28.1) |
| 12- | *Lactobacillus salivarius* | 5 | 7 | 12(35.3) | 3 | 4 | 7(23.3) | 19(29.7) |
| 13- | Unidentified organisms | ND* | ND* | - | 1 | 6 | 7(23.3) | 7(10.9) |
| 14- | *Staphylococcus aureus* | 1 | 1 | 2(5.9) | 4 | 3 | 7(23.3) | 9(14) |
| 15- | *Enterobacter aerogenes* | ND* | ND* | - | 1 | 5 | 6(20) | 6(9.4) |
| 16- | *Streptococcus sanguis* | 3 | 3 | 6(17.6) | 2 | 4 | 6(20) | 12(18.8) |
| 17- | *Fusobacterium nucleatum* | 5 | 3 | 8(23.5) | 1 | 5 | 6(20) | 14(21.9) |
| 18- | *Campylobacter ureolyticus* | ND* | ND* | - | 3 | 3 | 6(20) | 6(9.4) |
| 19- | *Klebsiellapneumoniae* ssp *pneumoniae* | 3 | 3 | 6(17.6) | 3 | 3 | 6(20) | 12(18.8) |
| 20- | *Leuconostoc mesenteroides* | ND* | ND* | - | 3 | 2 | 5(16.7) | 5(7.8) |
| 21- | *fusobacteriumnecrophorum* | ND* | ND* | - | ND* | 5 | 5(16.7) | 5(7.8) |
| 22- | *Proteus mirabilis* | ND* | ND* | - | 4 | 1 | 5(16.7) | 5(7.8) |
| 23- | *Serratiamarcescens* | ND* | ND* | - | ND* | 5 | 5(16.7) | 5(7.8) |
| 24- | *Streptococcus oralis* | 1 | 4 | 5(14.7) | ND* | 4 | 4(13.3) | 9(14) |
| 25- | *Streptococcus pyogenes* | ND* | ND* | - | 2 | 2 | 4(13.3) | 4(6.3) |
| 26- | *Citrobacterfreundii* | ND* | ND* | - | ND* | 3 | 3(10) | 3(4.7) |
| 27- | *Peptostreptococcus micros* | 3 | 3 | 6(17.6) | 1 | 2 | 3(10) | 9(14) |
| 28- | *Raoultellaornithinolytica* | ND* | ND* | - | 2 | ND* | 2(6.7) | 2(3.1) |
| 29- | *Sphingomonaspaucimobilis* | ND* | ND* | - | 1 | 1 | 2(6.7) | 2(3.1) |
| 30- | *Streptococcus infantarius* ssp. infantarius | 4 | 2 | 6(17.6) | 2 | ND | 2(6.7) | 8(12.5) |
| 31- | *Klebsiellaoxytoca* | ND* | ND* | - | 1 | 1 | 2(6.7) | 2(3.1) |
| 32- | *Pseudomonas fluorescens* | ND* | ND* | - | ND* | 1 | 1(3.3) | 1(1.6) |
| 33- | *Staphylococcus epidermidis* | 6 | 4 | 10(29.4) | ND* | ND* | - | 10(15.7) |
| 34- | *Lactococcuslactis ssp. cremoris.* | 6 | 4 | 10(29.4) | ND* | ND* | - | 10(15.7) |
| 35- | Bifidobacterium spp. | 1 | 5 | 6(17.6) | ND* | ND* | - | 6(9.4) |
| 36- | *Lactobacillus gasseri* | 4 | 2 | 6((17.6) | ND* | ND* | - | 6(9.4) |
| 37- | *Staphylococcus saprophyticus* | 3 | 2 | 5(14.7) | ND* | ND* | - | 5(7.8) |
| 38- | Bacillus spp. | 3 | 2 | 5(14.7) | ND* | ND* | - | 5(7.8) |
| 39- | *Lactobacillus plantarum* | 2 | 1 | 3(8.8) | ND* | ND* | - | 3(4.7) |
| 40- | *Actinomycesnaeslundii* | 2 | ND* | 2(5.9) | ND* | ND* | - | 2(3.1) |
| 41- | *Streptococcus pseudoporcinus* | ND* | 1 | 1(2.9) | ND* | ND* | - | 1(1.6) |

**N:** total number of participants (healthy/diseased implant, **Tn:** total number of microbial species in healthy or diseased implant, **No.:** number of isolates in a given microbe, **ND*:** not detected, **-** : 0(0%).

worth noting that some isolates showed a common association in both groups (*i.e.* test and control groups; N=64) as follows, 23(36%) *Streptococcus thoraltensis*, 23(36%) *Escherichia coli*, 19(29.7%) *Lactobacillus salivarius*, 18(28.1%) *Staphylococcus hominis*, 17(26.6%) *Veillonella* spp., 15(23.4%) *Streptococcus mutans*, 14(21.9%) *Candida albicans*, 14(21.9%) *Fusobacterium nucleatum*, 12(18.8%) *Streptococcus sanguis*, 12(18.8%) *Klebsiella pneumoniaes* spp *neumoniae*, 9(14) *Staphylococcus aureus*, 9(14) *Streptococcus oralis*, 9(14) *Peptostreptococcus micros*, 8(12.5%) *Streptococcus infantarius* ssp. infantarius.

The peri-implant microflora in healthy dental implants are mainly populated by Gram-positive cocci and non-motile bacilli and a limited number of Gram-negative anaerobic species (Mombelli *et al*, 1987; Bower *et al*, 1989). Whereas the shift to peri-implantitis is associated with increased presence of cocci, motile bacilli (Pontoriero *et al*, 1994). Deepened peri-implant pocket resulted from peri-implantitis lead change of habitat with low $O_2$ conditions which in turn does not support the growth of aerobic bacteria. Regarding to peri-implantitis, Culture-based techniques revealed the emergence of Gram-negative, facultative anaerobic species at peri-implantitis sites as well as *Candida* spp. (Alcoforado *et al*, 1991) and *Staphylococci* (Charalampakis *et al*, 2012). Aerobic Gram-negative bacilli include two wide and distinct categories; bacteria that ferment lactose and belong to the family Enterobacteriaceae (i.e. *E. coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*) and non-enteric rods that don't ferment lactose (i.e. *Pseudomonas aeruginosa*, *Acinetobacter baumannii*). Charalampakis *et al* (2012) demonstrated the presence of aerobic Gram-negative bacilli at moderately heavy or heavy growth in 18.6% of patients with peri-implantitis.

In general, existing data on the subgingival plaque composition of peri-implantitis have been detected in samples of peri-implantitis lesions, such as *Enterobacter cloacae*, *Enterobacter aerogenes*, *Pseudomonas* spp, *S. aureus* and *Candida* spp. (Leonhardt *et al*, 1999; Persson *et al*, 2010; Mombelli and Décaillet, 2011; Persson and Renvert, 2014; Rams *et al*, 2014).

Lactic acid bacteria (LAB) that function as probiotics are well known for their beneficial effects on humans and animals (Marteau and Rambaud, 1993; Naidu *et al*, 1999; Ljungh and Wadstrom, 2006). However, many authors have suggested that some LAB strains exhibit cariogenic activity (Harper and Loesche, 1984; Bradshaw and Marsh, 1998; Matsumoto *et al*, 2005). This may explain the percentage of (36.7%) *Lactobacillus acidophilus* among test group.

However, the dominant prevalence of lactic acid bacteria among healthy implant is attributed to their beneficial effects by competing with harmful bacteria associated with periodontal diseases. Probiotics (such as *Lactococcus* spp., *Bifidobacterium* spp.) stimulate dendritic cells (antigen presenting cells) resulting in expression of T-helper cell 1(Th1) or T-helper cell 2 (Th2) response, which modulates immunity. Probiotics enhances innate immunity and modulate pathogen induced inflammation through "Toll-like receptors" on dendritic cell. Intracellular pathogens are phagocytosed by Th1 response, while extracellular pathogens are taken care by Th2 response. Probiotics can mimic response similar to a pathogen but without periodontal destruction (Llewellyn and Foey, 2017).

## CONCLUSION

Significant diversity was observed between peri-implant patients microbiota and healthy microbiota in different ages and both genders.

## ACKNOWLEDGMENTS

Grateful thanks to the periodontist of some private dental clinics for their assistance in providing the samples. We thank the participants (diseased and healthy people) in the study. Also, we show gratitude to the Department of Biology, College of Science, Mustansiriyah University (http://uomustansiriyah.edu.iq/), Baghdad for advice and support.

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